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37 C.F.R. 1.8

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June 21, 2004
Date

Steven L. Highlander

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Dennis E. HALLAHAN *et al.*

Serial No.: 08/540,343

Filed: October 6, 1995

For: METHODS AND COMPOSITIONS FOR VIRAL ENHANCEMENT OF CELL KILLING

Group Art Unit: 1632

Examiner: S. Priebe

Atty. Dkt. No.: ARCD:194/SLH

SECOND SUPPLEMENTAL INVENTORS' DECLARATION UNDER 37 C.F.R. §1.131

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

We, the undersigned, do declare that:

1. We are citizens of the United States and named inventors on the above-captioned application.

2. One or more of the present inventors, or person(s) acting under their direction and control, were actively engaged in activities in this country relating to a reduction to practice of

the claimed invention from prior to June 23, 1994 to the filing date of the above-captioned application.

3. Attached to this declaration are redacted notebooks evidencing activity from about July 31, 1994 to October 6, 1995. Previously submitted materials showing work conducted prior to June 23, 1994.

4. Applicants have been unable to identify notebooks containing experimental results from the period of June 23, 1994 to July 31, 1994. However, during this time frame, the present inventors were discussing the present invention in the context of planning experiments, ordering of materials, or preparing to conduct experiments on at least a weekly (if not daily) basis in the period of time between June 23, 1994 and July 31, 1994. Thus, taken together, the evidence of record reflects continuous activity from before June 23, 1994 to applicants' filing date of October 6, 1995.

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

May 24, 2004	Ralph Weichselbaum
Date	Dr. Ralph Weichselbaum
Date	Dr. Dennis Hallahan
Date	Dr. Gregory Sibley
Date	Dr. Donald Kufe
Date	Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date	Dr. Ralph Weichselbaum
5/26/04 Date	Dennis Hallahan Dr. Dennis Hallahan
Date	Dr. Gregory Sibley
Date	Dr. Donald Kufe
Date	Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date	Dr. Ralph Weichselbaum
Date	Dr. Dennis Hallahan
6/18/04 Date	Dr. Gregory Sibley
Date	Dr. Donald Kufe
Date	Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date	Dr. Ralph Weichselbaum
Date	Dr. Dennis Hallahan
Date	Dr. Gregory Sibley
5/25/04 Date	Donald W. Kufe Dr. Donald Kufe
Date	Dr. Bernard Roizman

5. We hereby declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the referenced patent application or any patent issued thereon.

Date	Dr. Ralph Weichselbaum
Date	Dr. Dennis Hallahan
Date	Dr. Gregory Sibley
Date	Dr. Donald Kufe
June 10 2004 Date	Dr. Bernard Roizman

W/O Dates

Received from Joany Chou (
- SK-N-SH human neuroblastoma cell cultures
  medium = 10% FCS in DME
- R3616 (HSV 1 (γ34.5)) titer = 6×10^9 PFU/ml
- R 899-6 (R3616 + TNF) titer ~ 6×10^9 PFU/ml (titered in Rei's lab)

Gave Joany Chou ( )
- AT cells & medium
- SCC 61 cells & medium

Vero cells (medium = 5% FCS in DME) received from Elena

199V media (vms) received 500 cc. Order from Gibco

ATCC HTB 14 **U-87 MG** **(Glioblastoma, astrocytoma, grade III, human)**

Current medium for propagation: Eagle's MEM with non-essential amino acids, sodium pyruvate, 1 mM and Earle's BSS, 90%; fetal bovine serum, 10%

This is one of a number of cell lines derived from malignant gliomas (See also ATCC HTB 16, 17) by J. Ponten and associates from 1966-69 (Acta Pathol. Microbiol. Scand. *74:* 465-486, 1968; Hum. Hered. *21:* 238, 1971). Cultures were established as explants on grid-supported lens paper or gelatin foam with Eagle's minimum essential medium and 10% bovine calf serum as the culture fluid. Trypsinization of the outgrowth or cells attached to the vessel floor with subsequent transfer to standard vessels in growth medium permitted cell line development. A culture at passage 108 was deposited by J. Ponten in July, 1973. Mycoplasma contamination was eliminated in September, 1975.

ATCC HTB 14 (continued)

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-44; Sex-Female; Race-Caucasian; Blood Type-A^+.
Grown as: Monolayer; transferred 1:5 weekly.
Morphology: Epithelial-like.
***In Vitro* Cytopathology:** (P120) Consistent with glioblastoma.
Nude mouse: Produces malignant tumor consistent with glioblastoma.

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 122.
Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.
Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	2	3	1	15	21	7	1
Chromosomes:	40	41	42	43	44	45	47

The stemline chromosome number is hypodiploid, the 2S component occurring at 5.4%. Nine markers [t(1q;?3), t(1p22q), t(6p?11q-), t(6q?7p), t(7q;?), del(12q), t(20;1p;9q), t(?8p;?), and M1] were common to most S metaphases. Neither HSR's nor DM's were detected. The line was originated from a female patient. However, all S metaphases were monosomic for the X chromosome.

Viability: 93%.
Culture Medium: Eagle's minimum essential medium with non-essential amino acids, sodium pyruvate and Earle's BSS, 85%; fetal bovine serum, 15%; antibiotic-free.
Isoenzymes: Me-2, 1; PGM_3, 1; PGM_1, 2; ES D, 1; AK1, 1; GLO-1, 1; G6PD, B.
Phenotype Frequency Product: 0.0017.
Sterility: Tests for mycoplasma, bacteria and fungi were negative.
Species: Confirmed as human by isoenzyme analysis.
Note: This material is available under the conditions that you will not use it for commercial purposes or distribute it to third parties. Please see pages xv and xvi for the form required.
Price Code: J

ATCC HTB 15 **U-118 MG** **(Glioblastoma, human)**

Current medium for propagation: Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%.

This line is one of a series derived by J. Ponten and associates as discussed under ATCC HTB 14, 16 and 17 (Acta Pathol. Microbiol. Scand. *74:* 465-486, 1968). The source tumor was described as a grade III astrocytoma-glioblastoma with one area resembling an epdendymoblastoma. Cytoplasmic granulation was striking and astroblasts with neurofibrils were observed. Spongioblasts were abundant in culture and were not affected by frequent subcultivation.

A culture at passage 416 was provided originally by J. Ponten. Progeny transferred to the ATCC in 1982 were found to be contaminated with mycoplasma. The infection was cured in 1987 by treatment with BM cycline over a 6-week culture period.

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-50; Sex-Male; Race-Caucasian; Blood Type-A^+.
Grown As: Monolayer; transferred 1:3.5 weekly.
Morphology: Mixed.
Karyology: Hypopentaploid to hyperpentaploid with abnormalities including breaks (P419).
***In Vitro* Cytopathology:** All spindle giant cells, malignant.
Nude mouse: Yields pleomorphic malignant tumor consistent with glioblastoma multiforme invading muscle.
HLA Cell Line Phenotype: AW 24, 28; B12, W47 (Pollack, *et al.).*

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 443.
Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.
Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	1	1	3	1	2	4	1	4	1	3	4	2	3	3	5	1	2	2	2	1	1	2	1
Chromosomes:	71	99	102	103	104	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	123	125

ATCC CRL 1690 T98G (Glioblastoma, human)

†**Passage Frozen:** 427. **Current medium for propagation:** Eagle's MEM with non-essential amino acids, 1.0 mM sodium pyruvate and Earle's BSS, 90%; fetal bovine serum, 10%. **Additional Information:** This line was derived by L. Hayflick from a glioblastoma multiforma tumor from a 61-year-old male Caucasian. T98G has an indefinite lifespan and is anchorage-independent, but can enter a viable G1-arrested state when crowded or deprived of serum. These cells should be useful for studies on the mechanisms for cessation of proliferation in quiescent cells, and for studies requiring cells synchronized in G1 phase. This is a hyperpentaploid human cell line. The modal chromosome number should be around 128 to 132. The rate of cells with higher ploidies was 1.39%. Fourteen to 16 marker chromosomes were common to most cells. **Reference:** J. Cell. Physiol. *99:* 43-54, 1979. **Submitted by:** G.H. Stein, University of Colorado, Boulder, CO. Price Code: J

ATCC CRL 1691 C7 **(Mouse hybridoma, anti LDL receptors)**

Complete description appears in the Hybridoma Section of the Catalogue (pp. 333-349).

ATCC CRL 1692 HISM **(Smooth muscle, jejunum, human)**

†**Passage Frozen:** 12; PDL 16. **Current medium for propagation:** Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%. **Additional Information:** This cell line was derived from the *muscularis propria* of the jejunum of a normal 35-year-old female patient. It synthesizes collagen and contains actin stress fibers. It contracts in response to the C-terminal octapeptide of cholecystokinin. **Reference:** Proc. Soc. Exp. Biol. Med. *176:* 503-507, 1984. **Submitted by:** M.F. Graham and R.F. Diegelmann, Medical College of Virginia, Richmond, VA. Price Code: J

ATCC CRL 1693 NFS-5 C-1 **(Pre-B lymphoblast, mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 μg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. *129:* 751-758, 1982). **Additional Information:** This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-2SM ecotropic murine leukemia virus. The cells bear a pattern of markers consistent with identification as large pre-B lymphoblasts (*i.e.*, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB–, sIg–, Ia–, and Ly-1+). The cells spontaneously produce both ecotropic and mink cell focus-forming viruses. **Reference:** J. Immunol. *133:* 744-753, 1984. **Submitted by:** W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1694 NFS-70 C-10 **(Pro-B lymphoblast, mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 μg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. *129:* 751-758, 1982). **Additional Information:** This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-NS-7 ecotropic murine leukemia virus. The cells bear a pattern of markers suggestive of pro-B lymphoblasts (*i.e.*, Mac-1+, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB–, sIg–, Ia–, and Ly-1+). The cells appear to be of a very early stage of commitment to B-cell differentiation. **Reference:** J. Immunol. *133:* 744-753, 1984. **Submitted by:** W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1695 NFS-25 C-3 **(Pre-B lymphoblast, mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 μg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. *129:* 751-758, 1982). **Additional Information:** This line was derived from a spontaneously arising lymphoma in an NFS.C58v-1 mouse. The cells bear a pattern of markers consistent with identification as pre-B lymphoblasts (*i.e.*, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB–, sIg–, Ia–, and Ly-1+). **Reference:** J. Immunol. *133:* 744-753, 1984. **Submitted by:** W.J. Davidson, and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1696 McCoy **(Mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Eagle's MEM with non-essential amino acids and Earle's [illegible] 90% fetal bovine serum, 10%. **Additional Information:** Little descriptive information about the origin of the McCoy [illegible] in the literature. They were first mentioned by Pomerat, *et al.* (Z. Zellforsch. *47:* 158-174, 1957). The cells [illegible] have originated from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis. [illegible] showed that McCoy cells (designated McCoy A) were indeed human cells. However, another subline [illegible] was, in fact, of mouse origin and possessed marker chromosomes characteristic of strain L mouse [illegible] to be human, but which actually are mouse cells, have been disseminated from laboratory [illegible] Initial interest in McCoy cells followed the demonstration by Gordon and Quan (Proc. [illegible] 1965) and Gordon, *et al.*, (Appl. Microbiol. *23:* 123-129, 1972) that ionizing radiation [illegible] of McCoy cells to infection by chlamydia strains. A culture of the so-called [illegible] Disease Control, Cell Culture Department, Atlanta, GA in March, 1984. [illegible] not available. The cells have been used to propagate laboratory strains [illegible] The cell line has been satisfactory for chlamydia growth for at [illegible] Price Code: J

[illegible] receptors)

[illegible] (pp. 333-349).

samples

									TNF pg/ml cell	pg/ml sup
~~SQ~~	~~MOI 5~~	~~XR 2h~~	~~extract 24h~~	SQ20B	MOI 5	extract 24h	①	Ø	54	
~~HS~~	~~"~~	~~"~~	~~"~~		"			2h	63	255
~~SQ~~	~~MOI 0.1~~	~~XR 2h~~	~~"~~		"			6h	54	240
~~HS~~	~~"~~	~~"~~	~~"~~		"			24h	62	194
~~SQ~~	~~MOI 5~~	~~XR 6h~~	~~"~~	SQ20B	MOI 0.1	"		Ø	③ 200	278
~~HS~~	~~"~~	~~"~~	~~"~~					2	134	② 429
~~SQ~~	~~0.1~~	~~"~~						6	16	312
~~HS~~	~~"~~	~~"~~						24	7	304
	~~5~~	~~"~~	~~"~~	HS	MOI 5	"		Ø	③ 33	1212
	~~"~~	~~XR 24h~~			"			2	11	② 1407
	~~0.1~~	~~"~~			"			6	14	1029
		~~"~~			"			24	3	1119
		~~"~~	~~"~~	HS	MOI 0.1			Ø	257	1240
					"			2	② 477	1154
					"			6	480	1193
					"			24	① 60	1100

median of
1 240
2 255
3 240
4 194
" 5 278
6 429
7
" 8
9
10
" 11
12
13
14
15
16

x .2 ml = pg/10^7 cells
② 2h is best
① SQ killed
HS not ? ?
— titrate down virus, ↓ time (<24°), ↓ serum
[illegible] MOI [illegible] SQ

10^8 PFU 10^{-3} 10^{-6}

HSV-R (425-TNF) 5×10^8/ml (particles) MOI = 5 ∴ $10^{[illegible]}$

HSV-F Ø 10^8/ml

Cells (TNF)

SQ 20B HS 638 (glioma)

1) SQ more sensitive
2) PFU & time depend cytotoxic in each
3) 0.1 PFU more than enough
4) 24° may be too long for induction since virus cytolytic

Titrate down

1) PFU of .02 gave 1000 pg/ml / .002 240-410 pg/ml
2) no induction

Survival

1) both Ø & TNF cytolytic
2) both enhanced XR kill even at 1 day

"

Ø

- cell density dependent cytotoxicity
  cytotoxicity transferred in medium
  PFU .001 - little cytotox but (+) enhance of XR kill

Program

low PFU → hardly infected

copy pg83

Decanted CM

confluent x 4 days unfed
1/200 HSV ± XR (3 Gy) 6 hrs later
18hr decant & feed
80% or 20% of each onto confluent cells

<u>all 1/200 HSV</u>

Ø fed	0	*
Ø fed → subculture	40/100 122/200 200/500	
3Gy fed	0	
3Gy fed → subculture	90/500 157/10 ~200/10⁴	*
20% Ø CM	0	
20% Ø CM → subculture 24°	79/200 100/100 236/500	cell cycle effect?
20% 3Gy	0	
20% 3Gy → subculture 24°	200 100/500 250/10³ >1000/10⁴	
80% Ø	confluent	
80% Ø → sub 24°	61/200 43/100 162/500	
80% 3Gy	confluent	*
80% 3Gy → sub 24°	253/500 >1000/10⁴ 500/10³	
24h CM Ø → ? sub 24° ? ± XR		
24h CM 3Gy → ? sub 24° ? ± XR		
48h CM Ø	0/200 0/10³ 0/500	*
48h CM 3Gy	0/200 0/10³ 0/500	

? toxin vs depletion vs serum vs artifact (over trypsin to confluent)

1) ? does cell density effect PE 2) ? is a new protein made after treatment

	PE	3 Gy
40λ	300, 500, 10^3, 2×10^3, 5×10^3, 10^4 →	"
4λ	"	"

} on shaker for 1 hr in 1 ml medium

(2) SQ 20B 10^5 in T-75 (2)

(3) SQ 20B 5×10^5 on p100 – 4 dishes → ^{35}S-methionine

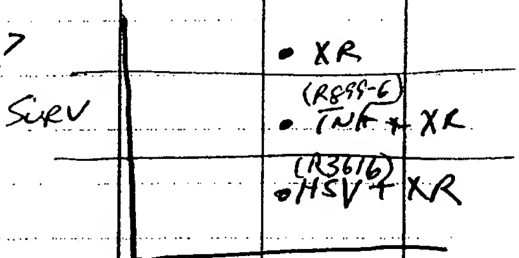
	PE						3 Gy					
40	30/300	30/500	180/1000	200/2000	25/5000	15/10^4	20/300	20/500	30/1000	100/2000	10/5000	25/10^4
4	30/300	50/500	100/1000	120/2000	780/5000	20/10^4	10/300	25/500		0/2000	40/5000	40/10^4

(1) What is the relative infectivity of HSV 3616 in SQ-20B compared to Vero cells?

(2) What level of ~~tumor~~ viral innoculum can nude mice tolerate?

in vitro (3) At what PFU is TNF production maximized?
At what time point is TNF production maximized?
Compare wt HSV I to HSV 3616 to HSV 899-6
~~Does RT [illegible] TNF production~~

(4) How does RT affect cytotoxicity?
where does HSV alone fall?



(5) Does supernatant sensitize? How about supernatant to Vero ce[lls]
Does supernatant retain infectivity or toxicity?

(6) Is TNF produced c̄ viral infection s̄ RT?

(7)

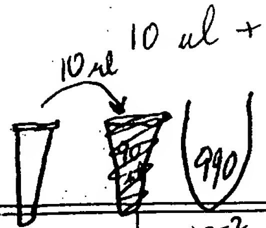
AMPAD · EFFICIENCY LINE® 22-206

	1	2	3	4	5	6	7	8	9

1 Any reason to keep working c̄ RB99-6 ?

2

100 ul (10^-1^) + 900 ul (10^-2^) ≠ 1 ml of 10^-2^



100 ul (10^{-1}) + 900 ul (10^{-2}) ≠ 1 ml of 10^{-2}

Need: 199V   Glass bottles autoclaved

199Ö

Human γ globulin (need to order)

6 T-25's for each cell line

SQ-20B

SK-N-SH

Vero

Need Vero cells from Elena

Reagents

Human γ-glob.

199 V medium

PBS

methanol

Giemsa stain (1X)

Distilled H_2O

Biologicals

6 T-25's of confluent cells p

cell type

Equipment

Dilution tubes 1!

Need from Elena

- Vero cells
- 199 V media (250

Need to order

- Gamma globuli

ARMOR Pharm

3 Versene

3 Trypsin

V ½ of V + ½ of T = 6 ml   4 of media

incubate

count c̄ cytometer

? spin 5/8 for 5 mins

? resuspend c̄ 5% FCS in DME (25 ml)

? 7 #cells per T-25 → incubate (? total vol)

? freeze soln   20% FCS   10% DMSO or Glycerol

? stock sol'n

13 Gy = 9 ~~36~~ 28

3 ~~52~~

12:28

10^{-5} 10^{-6} 10^{-7} 10^{-8}

SQ

10^{-5}

10^{-6}

10^{-7}

10^{-8}

10^{-5} 10^{-6} 10^{-7} 10^{-8}

SQ-20B

SK-N-SH

Vero   —   —

—   —

Doubling times

SQ-20B - 10^5 plated in T-25. On d 4 = 50% confluent

SK-N-SH ~ 10^5 plated in T-25. On d 4 = 80-90% confluent

Vero cells -

MEDIUM 199/1% calf serum(pH7.6-7.9)

Application: virus propagation and titration.

Preparation: 1X 199v

room T° sterile glass distilled water	440mls
10X 199 Hank's BSS w/o L-glutamine(Hazleton)	50mls
1000X Pn/Strept	0.5mls
heat inactivated bovine calf serum(Colorado Serum)	5mls
100X L-glutamine(Sigma)	5mls
7.5%(w/v)$NaHCO_3$(Sigma)	9.35mls

(1) $NaHCO_3$ must be added last.

(2) $NaHCO_3$ concentration must be 1.4g/l in the 1X formulation. The color of the 1X medium after addition of $NaHCO_3$ should be cherry red. If necessary, add 6N NaOH dropwise to achieve the desired color. DO NOT increase amount of $NaHCO_3$.

(3) Store at 4°C; shelf life 4-6 weeks.

Preparation: 1X 199o

1X 199v	500mls
pooled human gamma globulin	0.5mls

To 500mls 1X 199v asceptically add 0.5mls(0.1%) pooled human immunoglobulin.

Shelf life: suggest preparing fresh.

Medium 199 = Gibco Cat No 11181-021
10 X
c̄ Hanks salts
c̄ L-glut
s̄ $NaHCO_3$

GIEMSA STAIN

Application: Solution used to stain viable cells; specifically used in cell culture for virus titration assay.

Source: Sigma

Preparation: 10X GIEMSA

Time Element: 16 day period

giemsa powder..............5 grams
glycerol...................500mls
methanol...................500mls

Day1

(1) Put giemsa and glycerol into a flask which has a layer of glass beads .

(2) Place flask on a shaker at 37^{o}C; shake overnight.

Day2

(3) Remove flask from 37^{o}C and add methanol.

(4) Mix(with stir bar)at room temperature .

(5) Place in dark for 2 weeks(This can be achieved by completely wrapping flask with foil.) at room temperature.

Day16

(6) Filter solution with Whatman #1 paper.

(7) Store at room temperature. No known expiration.

Strengths:

Stock: 10X

Working: 1X (Prepare day of use by diluting 10X stock 1:10 with glass distilled water; eg: 1ml 10X + 9mls water).

use 7.5ml (10 x stock) ~~in 50ml wt~~ + 42.5ml dH_2O

SQ20B : 3616 vs 899-6 , 10^{-1} vs 10^{-3} MOI

SIBLEY

1	2	3	4	5	6	7	8	9

EFFICIENCY LINE® 22-206

AMPAD

Purpose

- Quantify cell Killing c̄ R3616 & R899-6 vs. no virus following 5 Gy RT.
- Does the TNF construct in R899-6 lead to additional cell kill over R3616?
- Quantify TNF production in serum and pellet after RT & infection.
- Compare cell killing ~~with~~ and TNF production with MOI of 10^{-1}, 10^{-3}.

Design

- Fixed Variables - Cell type: SQ-20B
  RT dose: 5 Gy single fraction
  Interval infect → RT: 16 hrs
  Interval RT → subculture: 4 hrs

- Study Variables - Virus: None, R3616, R899-6
  MOI: 10^{-1} PFU/cell, 10^{-3} PFU/cell

SIBLEY

Methods —

(1) Plate 10^6 SQ-20B cells / 60 mm dish.
Volume/dish = 3-4 ml 20% FCS in DME + P/S
Incubate to 95% confluency (overnight)
Label Plates: 10^{-1} MOI R3616 10^{-3} MOI R3616
(duplicate) 10^{-1} MOI R3616 10^{-3} MOI R3616
10^{-1} MOI R899-6 10^{-3} MOI R899-6
10^{-1} MOI R899-6 10^{-3} MOI R899-6
Ø virus
Ø virus ∴ need 10 plates + 1 extra to count cells

(2) Count a plate using hemocytometer = 1.12×10^6 cell
(3) Make dilution of viruses:
R3616 = 6×10^9 PFU/ml = 6×10^6 PFU/µl

10 µl → (90) → 10 µl → (90) → 10 → (90) → 10 → (90)
6×10^5 PFU/µl, 6×10^4, 6×10^3, 6×10^2

R899-6 = 6×10^9 PFU/ml also, ∴ make same dilution

(4) Change media on plates to 2% FCS in DME, 2 ml/pl
(5) Add virus (12^{noon})
For 10^{-1} MOI need $\frac{1.12 \times 10^5 \text{ PFU}}{6 \times 10^4 \text{ PFU/µl}} = 1.87$ µl of 6×10^4 dilution

For 10^{-3} MOI need $\frac{1.12 \times 10^3 \text{ PFU}}{6 \times 10^2 \text{ PFU/µl}} = 1.87$ µl of 6×10^2 dilution

(optimally want vol of viral innoculum ~ 2-3 µl)

SIBLEY

Methods (cont.) –

(6) Incubate 2 hrs, then add 2 more mls of 2% FCS (2 PM)

(7) Next am (11 AM) 5 Gy RT. (Carry dishes in sealed tupperware container). Use Cu-Al filter, 250 kV, 26 mamp, 50 cm plate-diaphragm distance. Time = 4' 48"

(8) Label eppendorf tubes for TNF assay samples = CM (conditioned media) & pellet for each dish.

(9) Label dilution tubes for each plate = 10^6, 10^5, 10^4, 10^3. Add 9 ml of media to each tube (except 10^6 tubes)

(10) Label 100 mm plates. Need 3 plates per original plate for plating dilutions = 10^4, 5×10^3, 10^3.
Add 10 cc of 20% FCS media to each plate

(11) Subculture dishes 4 hrs p̄ RT (3 pm). Do 4 at ✱ a time. Count 1 of each duplicate dish c̄ cytometer to resuspend to 10^6. (Counts were $\sim 37 \times 10^4$ cells/ml x 5 mls = resuspend in 1.85 mls) (Used 1 ml Versene + 1 ml Trypsin + 3 ml media = 5 ml for subculturing). Put samples on ice.

(12) Make dilutions of each sample $10^6 \rightarrow 10^5 \rightarrow 10^4 \rightarrow 10^3$

(13) Plate dilutions in 100 mm plates @ 10^4, 5×10^3 & 10^3

(14) Save 1 ml of supernatant after centrifuging as "CM" (conditioned media). Save 1 ml of 10^6 dilution as "P" (pellet) in eppendorf tubes, for TNF assay. Put in freezer.

(15) Incubate plates for ~ 1 week

(16) TNF assay - see next sheet

TNF-ELISA RESULTS

1	2	3	4	5	6	7	8	9
R3616		[TNF] pg/ml					[TNF] pg/ml	
10^{-3}	cm	0			10^{-1}	cm	0	
10^{-3}	cm	0			10^{-1}	cm	0	
10^{-3}	P	0			10^{-1}	P	0	
10^{-3}	P	0			10^{-1}	P	0	
R899-6		[TNF] pg/ml					[TNF] pg/ml	
10^{-3}	cm	0			10^{-1}	cm	~~25~~	32.7
10^{-3}	cm	0			10^{-1}	cm	~~25~~	32.3
10^{-3}	P	0			10^{-1}	P	~~110~~	51.5
10^{-3}	P	0			10^{-1}	P	~~170~~	56.6
Ø virus		[TNF] pg/ml						
	cm	0						
	cm	0						
	P	0						
	P	0						

cm = Conditioned media
P = pellet (resuspended @ 10^6 cells/ml)
10^{-3}, 10^{-1} = Viral MOI

ELISA

Standards

0 med.	1	9	17
15.7	2	10	
33.1	3	11	18
62.5	4	12	
125	5	13	19
250	6	14	
500	7	15	20
1000	8	16	

Put TNF in 1ml distilled H_2O

1. dilute standards in 0.5ml RD5 buffer (1:2)
2. Add standards (+ media only) 200ul to duplicate wells
3. Add 200ul of sample to each well, cover shake 1 hr.
4. Wash 3X by aspirating liquid, adding ~200ul of wash buffer
5. Add 200ul of conjugate A - shake ~30 min.
6. Wash again (make up A+B)
7. A 200ul A+B

1. 3616 10^{-3} cm
2.
3. 3616 10^{-3} P
4.
5. 3616 ~~899-6~~ 10^{-1} cm
6.
7. 3616 ~~899-6~~ 10^{-1} P
8.
9. 899-6 10^{-3} cm
10.
11. 899-6 10^{-3} P
12.
13. 899-6 10^{-1} cm
14.
15. 899-6 10^{-1} cm

17. Øvirus cm
18. ~~[illegible]~~
19. Øvirus P
20. ~~[illegible]~~

RESULTS (cont)

Colony counts

~~ØVirus~~

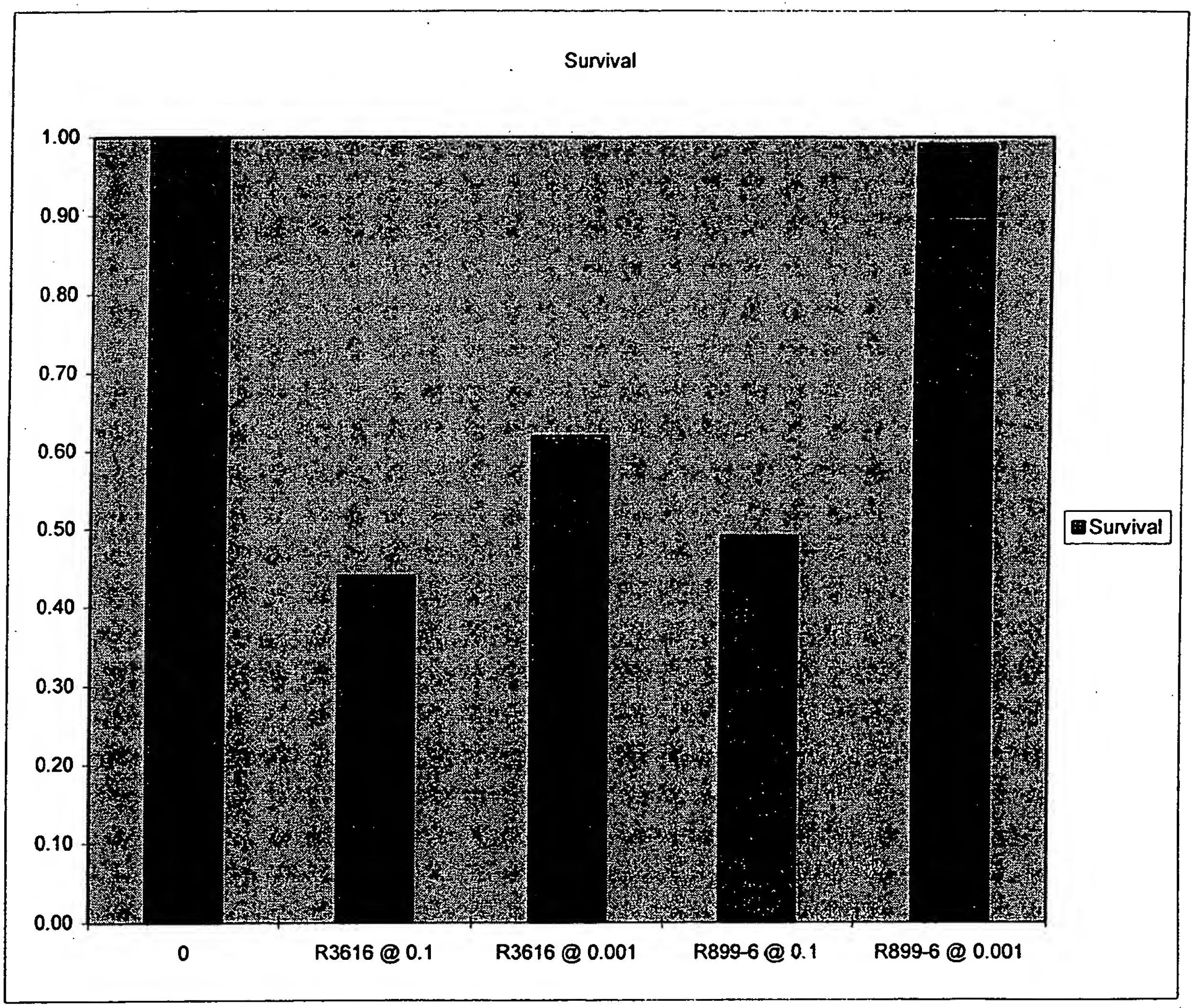
virus	MOI	# cells plated	count		*Approx Survival	Relative Efficac
Ø	0	10^3	144	> 176	35%	
"	"	"	208			
R 3616	10^{-1}	10^3	66	> 77.5	16%	
"	"	"	89			
"	10^{-3}	"	89	> 109	22%	
"	"	"	129			
R 899-6	10^{-1}	10^3	105	> 86.5	17%	
"	"	"	68			
"	10^{-3}	"	176	> 174.5	35%	
"	"	"	173			
"	"	5×10^3	700			

*(NOTE: No control plated, i.e. Ø virus - ØRT, ∴ can't determine plating efficiency)

If assume P.E. of 50%

	Results - Counts SQ20B				
RT	Virus	# Plated	Ave. Coun	P.E.	Survival
5	0	1000	176	0.176	1.00
5	R3616 @ 0.1	1000	77.5		0.44
5	R3616 @ 0.001	1000	109		0.62
5	R899-6 @ 0.1	1000	86.5		0.49
5	R899-6 @ 0.001	1000	174.5		0.99
Virus	Survival				
0	1.00				
R3616 @	0.44				
R3616 @	0.62				
R899-6 @	0.49				
R899-6 @	0.99				

SQ



Survival
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
R3616 @ 0.1
R3616 @ 0.001
R899-6 @ 0.1
R899-6 @ 0.001
Survival

Findings

1.) In SQ-20B:

a. No detectable TNF production @ MOI 10^{-3} $\bar{c}$ 899-6 (+5 Gy)

b. TNF detectable at MOI 10^{-1} (899-6 + 5Gy) with 55 pg/ml in pellet sample & 32 pg/ml in supernatent sample. (pellet sample = 10^{6} cells/ml)

c. Despite TNF production, R899-6 did not produce more cell killing than R3616 (@ MOI 10^{-1} + 5 Gy)

d. Both viruses caused increased cell killing at an MOI of 10^{-1}

e. R3616 appeared to give more effective cell killing than R899-6 or Ø virus at MOI of 10^{-3}.

The University of Chicago Departmental Purchase Order

Purchase Order Number
Z 849078
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
American Type Culture Collection
12301 Parklawn Dr.

Rockville (City) | MD (State) | 20852 (Zip Code)

Payment Terms ____ Delivery charge? ☐Yes ☐No

Telephone No. (800) 638-6597 FAX No. ____

Ship To
THE UNIVERSITY OF CHICAGO — Dept. Code: 1050
Radiation and Cellular Oncology (Department)
Michael Beckett (Attn:) G-03 (Room)
5830 S. Ellis Ave.
Chicago (City) | IL (State) | 60637 (Zip Code)

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; second copy should be sent to vendor; third copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with department code number from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ____ Payroll No. ____

Mahmud Ghaemi (Print Name) 2- (Ext.)

Account Code 5-25754-5400 | 8/8/94 (Date)

Order placed by phone? ☐No ☒Yes

Alonzo (Order placed with (name)) Date

Michael Beckett (Order placed by (name)) 2-9833 (Phone #)

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	Flask	ATCC HTB 14 U-87 MG Cell Line	75.00	75.00
		Lab Fee	35.00	35.00
		Pkg.-Fee	5.00	5.00
		Fed. Express	9.75	9.75
		Acct. # 2168		

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED

ORDER TOTAL 124.75

Form ZDPO 100 06/92

MOLECULAR DEVICES   [illegible] KINETICS MICROPLATE READER   PLATE #: 1

ASSAY :

DATE:

OPERATOR: SIBLEY   O.D. LIMIT: 3.000   TIME: ____ : ____ AM/PM

WAVELENGTH: 450nm - OPT 2   READ MODE: OPTICAL DENSITY   AUTO MIX: [illegible]

NOTES: CAL: [illegible]

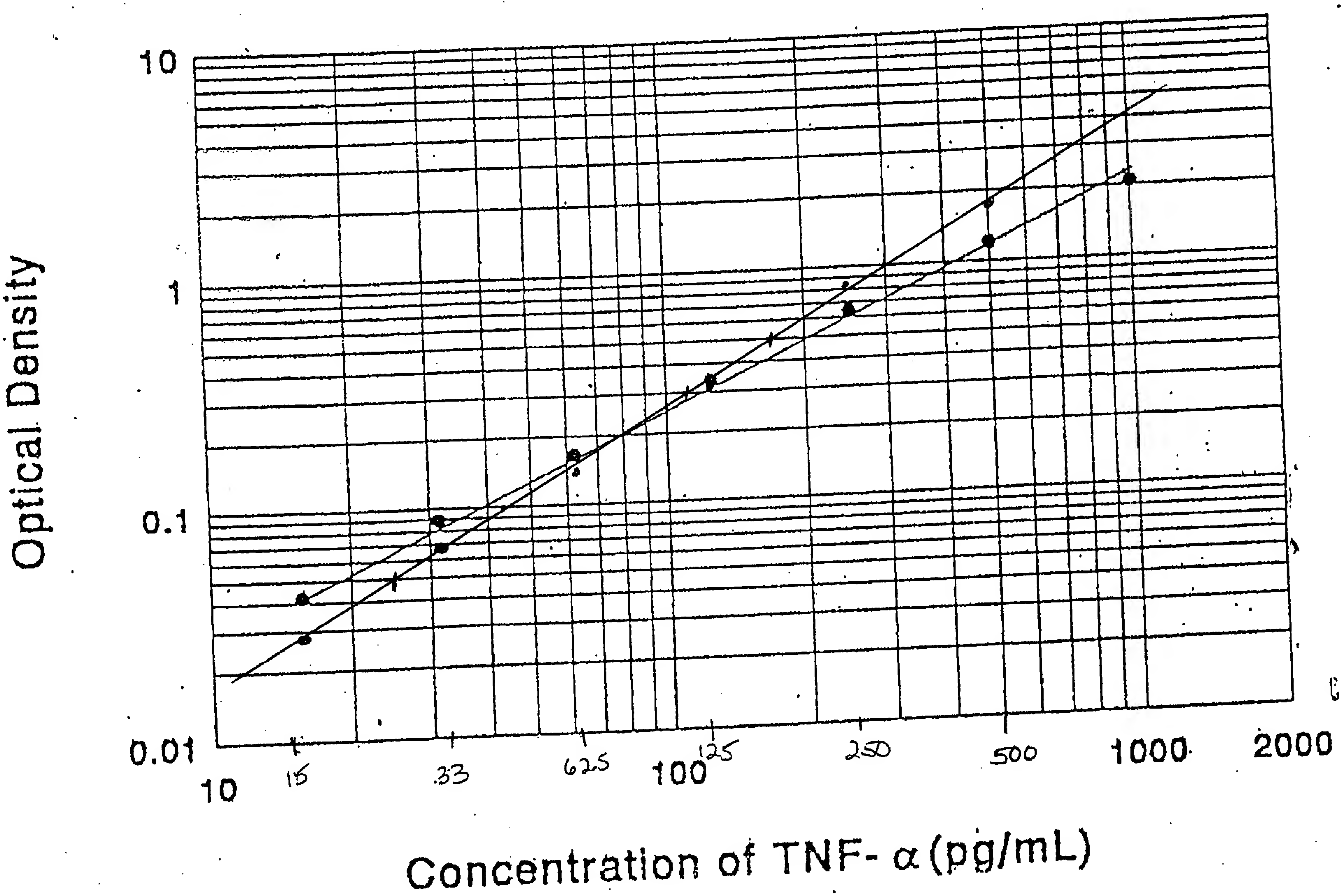
3616   899-6   Ovins

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
				0		0						
A	0.052	0.054	0.049	0.051	0.046	0.047	0.047	0.000	0.000	0.000	0.000	0.000
	.028	.027		~~050~~ 0		0	0					
B	0.080	0.078	0.045	0.050	0.045	0.048	0.048	0.000	0.000	0.000	0.000	0.000
	.069	[illegible]		0		0						
C	[illegible]	0.120	0.046	0.044	0.047	0.047	0.051	0.000	0.000	0.000	0.000	0.000
	.161	.158		0		~~.064~~ .009	0					
D	0.213	[illegible]	0.044	0.051	[illegible]	0.078	0.039	0.000	0.000	0.000	0.000	0.000
	.311	.326		0		.051						
E	[illegible]	[illegible]	0.048	0.048	0.106	0.090	[illegible]	0.000	0.000	0.000	0.000	0.000
	.879 .860	.844		0		.049	0					
F	0.927	0.896	0.046	0.047	[illegible]	0.101	0.047	0.000	0.000	0.000	0.000	0.000
	1.777	1.851		0		.120						
G	1.829	[illegible]	0.047	0.046	[illegible]	0.172	0.050	0.000	0.000	0.000	0.000	0.000
	1.814			0		.139	0					
H	*	*	0.049	0.050	0.185	0.197	0.050	0.000	0.000	0.000	0.000	0.000

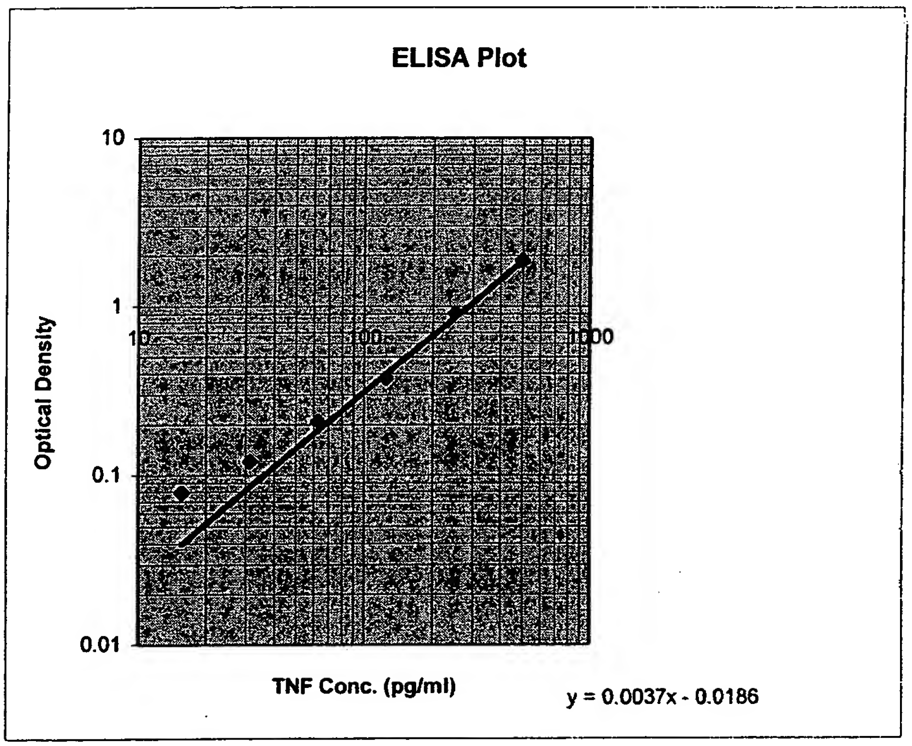
TNF-α Standard Curve

Diluent RD5



ELISA Plotting Worksheet

reading 1	reading 2	concentration	ave. reading
0.08	0.078	15.6	0.079
0.121	0.12	31.3	0.1205
0.213	0.207	62.5	0.21
0.363	0.392	125	0.3775
0.927	0.896	250	0.9115
1.829	1.903	500	1.866
0.106	0.099	32.7	0.1025
0.101	0.101	32.3	0.101
0.172	0.172	51.5	0.172
0.185	0.197	56.6	0.191



T986: 899-6 ~~only~~ @ MOI 10^{-1}
RT 0, 2, 5, 20 [TNF]

SIBLEY

Purpose
- Quantify killing of T986 (glioblastoma cells) with R899-6 virus and 0, 2Gy, 5Gy & 20 Gy RT.
- Determine TNF production (? inducibility) with varying RT doses by ELISA.

Design
- Fixed Variables - Cell type: T986
~~RT~~
Virus: R899-6 @ MOI of 10^{-1}
Interval infection → RT: 16 hrs
Interval RT → subculture: 6 hrs

- Study variables - RT dose: 0, 2Gy, 5Gy, 20Gy
Aliquot media for TNF: prior to RT, 30min, 1hr, 2h, 4h,
Virus: None, R899-6

4 x 2 x 2(dupl) = 16 plates

Methods - see

1. Plated 5×10^5 T98G (Glioblastoma)
2. Plate count = 7.5×10^5 cells
3. Dilutions made
4. Change media to MEM + P/S + 1% NaPyruvate + 1% ess a.a. + 2% FCS.
5. Add virus (3^{30} pm)

   for 10^{-1} MOI 894-6 need $\frac{7.5 \times 10^{4} \text{ ~~cell~~ PFU}}{6 \times 10^{3} \text{ PFU/ul}} = 12.5$ ul

6. Wait 2 hrs, add 2 more ml of 2% media 5^{30} pm
7. Irradiate 6^{45} - 7^{15}
8. Aliquot 0.5 ml media @ 30 mins = 7^{30}

   @ 1 hr = 8^{15}

   @ 2 hr = 9^{15}

   @ 4 hr = 11^{15}

9. Subculture @ 6 hr = 1^{15} (Don't subculture 20Gy plates)

   counts:

894-6	Ø virus
0Gy =	0Gy =
2Gy =	2Gy =
5Gy =	5Gy =
20Gy =	20Gy =

   * Save 1 ml of supernatant & pellet for TNF

10. Plate @ 10^3, 5×10^3, 10^4

TNF ELISA RESULTS

(Note: error in standards, ∴ old std curve used)

Cell type: T986 (Glioblastoma)
Virus: R899-6 @ moi of 10^{-1}

RT	Aliquot Time	Virus	[TNF]
0 Gy	30'	899-6	N/A
	1°		N/A
	2°		49
	4°		64
	6°		94
	Pellet (10^5/ml)		23 = 230 @ 10^6/ml
2 Gy	30'	899-6	59
	1°		67
	2°		(115)
	4°		78
	6°		73
	Pellet (10^5/ml)		25 = 250 @ 10^6/ml
5 Gy	30'	899-6	46
	1°		50
	2°		54
	4°		70
			101
	…et (10^5/ml)		24 = 240 @ 10^6/ml
20 Gy	30'	899-6	56
	1°		83
	2°		66
	4°		82
	6°		127
	Pellet (10^5/ml)		27 = 270 @ 10^6/ml

Controls (No Virus)

0 Gy
2 Gy
5 Gy
20 Gy
} No TNF

	reading 1	reading 2	concentration		ave. reading
	0.08	0.078	15.6		0.079
	0.121	0.12	31.3		0.1205
	0.213	0.207	62.5		0.21
	0.363	0.392	125		0.3775
	0.927	0.896	250		0.9115
	1.829	1.903	500		1.866
1°	0.181	0.181	53.9		0.181
	0.143	0.143	43.7		0.143
2°	0.226	0.24	68.0		0.233
	0.2	0.201	59.2		0.2005
4°	0.291	0.293	83.9		0.292
	0.369	0.362	103.8		0.3655
6°	0.066	0.067	23.0		0.0665
	0.069	0.063	22.9		0.066
	0.243	0.253	72.1	30'	0.248
	0.152	0.143	44.9		0.1475
	0.157	0.167	48.8	1°	0.162
	0.303	0.287	84.8		0.295
	0.532	0.517	146.8	2	0.5245
	0.175	0.173	52.1	6	0.174
	0.314	0.336	92.9	6	0.325
	0.203	0.195	58.8	4	0.199
	0.345	0.342	97.9	4	0.3435
	0.293	0.286	83.3	2	0.2895
	0.062	0.067	22.5		0.0645
	0.076	0.086	26.9		0.081

0.157	0.158	47.6	0.1575
0.142	0.146	43.9	0.144
0.148	0.151	45.4	0.1495
0.191	0.179	55.0	0.185
0.183	0.192	55.7	0.1875
0.177	0.17	51.9	0.1735
0.234	0.251	70.6	0.2425
0.231	0.238	68.4	0.2345
0.346	0.337	97.3	0.3415
0.371	0.36	103.8	0.3655
0.063	0.075	23.7	0.069
0.064	0.073	23.5	0.0685
0.182	0.203	57.1	0.1925
0.17	0.193	54.1	0.1815
0.208	0.247	66.5	0.2275
0.348	0.358	100.4	0.353
0.246	0.248	71.8	0.247
0.205	0.209	61.0	0.207
0.261	0.273	77.2	0.267
0.301	0.306	87.1	0.3035
0.457	0.448	127.3	0.4525
0.456	0.437	125.7	0.4465
0.083	0.079	26.9	0.081
0.085	0.079	27.2	0.082

1 STANDARDS		3 0 Gy		5	6	7	8	9
0	med	30'	"	6°	"	2°	"	
15	"	30'	"	6°	"	6°	"	
31	"	1°	"	P	"	6°	"	
62	"	1°	"	P	"	4°	"	
125	"	2°	"	$\boxed{2\ Gy}$ 30'	"	4°	"	
250	"	2°	"	30'	"	2°	"	
500	"	4°	"	1°	"	P	"	
1000	"	4°	"	1°	"	P	"	
$\boxed{5\ Gy}$								
LANE 9	10	11	12	13	14	15 $\boxed{ØV'NG}$	16	
30'	"	6°	"	2°	"	0Gy-S	"	
30'	"	6°	"	2°	"	0Gy-P	"	
1°		P°	"	4°	"	2Gy-S	"	
1°		P°	"	4°	"	2Gy-P	"	
2°		$\boxed{20\ Gy}$ 30'	"	6°	"	4Gy-S	"	
2°		30'	"	6°	"	4Gy-P	"	
4°		1°	"	P	"	6Gy-S	"	
4°		1°	"	P	"	6Gy-P	"	

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — PLATE #:

ASSAY : DATE: ____/____/____

OPERATOR: O.D. LIMIT: [illegible] TIME: ____:____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY AUTO MIX: OFF

NOTES: CAL: [illegible]

OPTICAL DENSITY

	1 (13)	2 (14)	3 (15)	4 (16)	5	6	7	8	9	10	11	12
A (−2°)	0.246	0.246	0.053	0.052	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000
B	0.205	0.203	0.053	0.052	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	[illegible]
C (−4°)	0.261	0.273	0.059	0.057	0.000	-0.000	[illegible]	[illegible]	[illegible]	0.000	[illegible]	[illegible]
D	0.301	0.306	0.058	0.060	-0.000	-0.000	-0.000	[illegible]	[illegible]	-0.000	[illegible]	[illegible]
E (−6°)	0.457	0.448	0.056	0.053	-0.000	-0.000	-0.000	[illegible]	-0.000	-0.000	-0.000	[illegible]
F	0.456	0.427	0.055	0.056	-0.001	[illegible]	[illegible]	[illegible]	[illegible]	-0.000	[illegible]	[illegible]
G (−P)	0.083	0.079	0.056	0.056	-0.001	-0.000	-0.001	-0.000	-0.000	-0.001	-0.000	-0.000
H	0.085	0.079	0.059	0.057	-0.001	-0.001	-0.000	[illegible]	[illegible]	-0.000	-0.000	[illegible]

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — PLATE #

ASSAY : DATE: ____/____/____

OPERATOR: O.D. LIMIT: [illegible] TIME: ____:____ AM/PM

WAVELENGTH: 450nm - OPT 2 READ MODE: OPTICAL DENSITY AUTO MIX: [illegible]

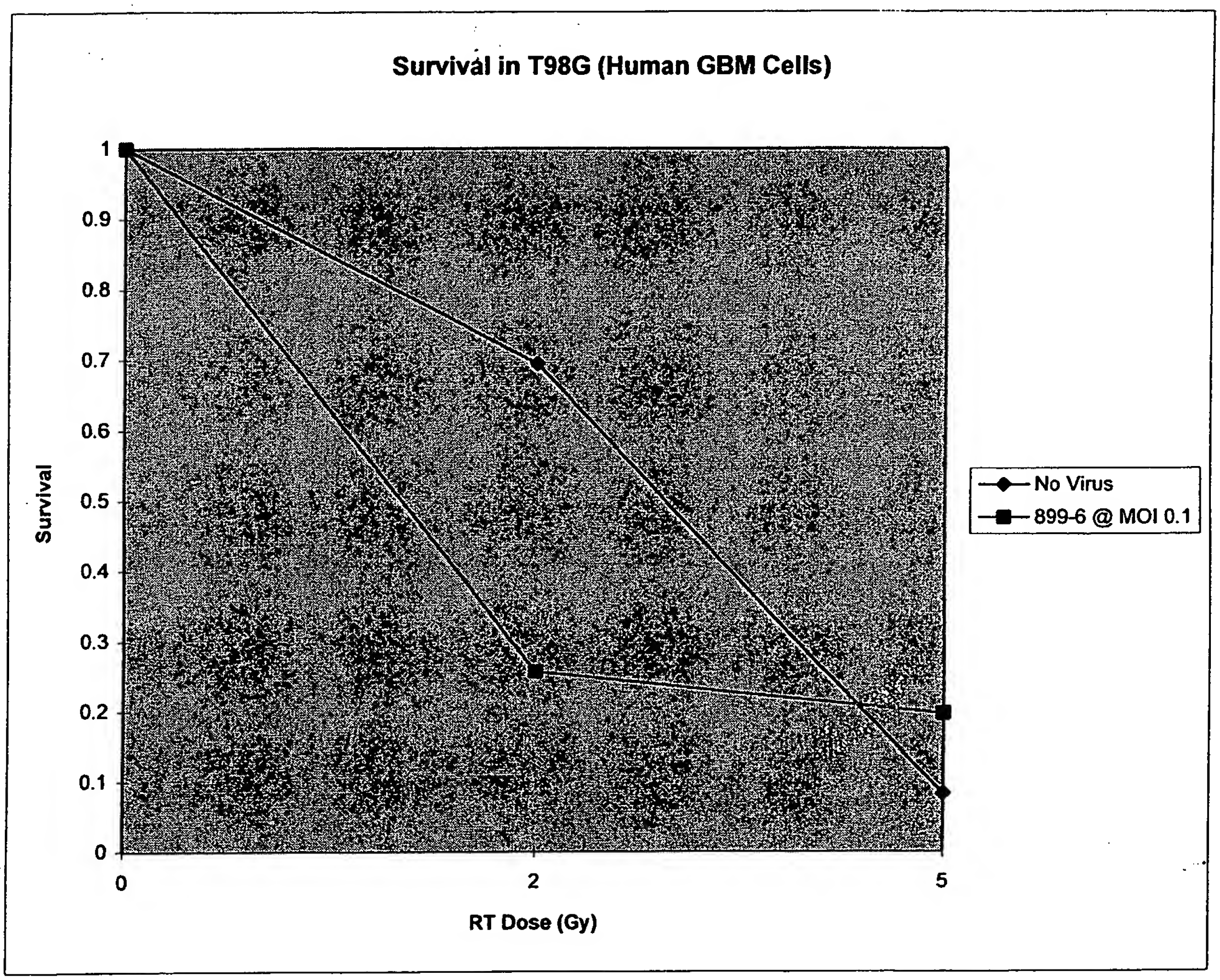
NOTES: CAL: [illegible]

OPTICAL DENSITY

0Gy 2Gy 5Gy 20Gy

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	0.000	-0.000 (30')	0.003	0.231 (6°)	0.252	~~0.812~~	~~[illegible]~~	0.127 (30')	0.138	0.318 (6°)	[illegible]
B	0.008	0.006	0.007	0.008	0.380	0.362	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C	0.004	0.001	0.007 (1°)	0.004	0.066 (−P)	0.067	[illegible] (6°)	0.330	[illegible] (−1°)	0.151	0.082 (−P)	0.075
D	0.000	0.007	0.003	0.007	0.059	0.052	[illegible] (4°)	0.198	0.191	0.179	0.064	0.072
E	0.002	0.005	0.051 (−2°)	0.181	0.243 (30' 2°)	0.253	0.345 (4°)	[illegible]	0.183 (−2°)	0.192	0.188 (30')	0.201
F	0.001	0.004	0.085	0.143	0.152 (1°)	0.143	0.293 (2°)	[illegible]	[illegible]	0.170	0.170	0.153

5^5/_{50}


Survival in T98G (Human GBM Cells)
Survival
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
5
RT Dose (Gy)
No Virus
899-6 @ MOI 0.1

Results- Count Plates T98G Cells

RT	Virus	# Plated	# Counted	#Counted	Average	P.E.	Survival	
0	0	1000	154	104	0.129	0.129	1	
		5000	tm	tm				
		10000	tm	tm				
2	0	1000	75	104	0.0895		0.693798	
		5000	tm	tm				
		10000	tm	tm				
5	0	1000	18	13	0.0155		0.120155	0.081783
		5000	20	44	0.0064		0.049612	
		10000	71	124	0.00975		0.075581	
0	899-6	1000	12	7	0.0095	0.015267	1	
	(MOI=0.1)	5000	68	139	0.0207			
		10000	156	156	0.0156			
2	899-6	1000	3	6	0.0045		0.294118	0.255991
		5000	13	17	0.003		0.196078	
		10000	25	60	0.00425		0.277778	
5	899-6	1000	4	2	0.003		0.196078	0.196078
		5000	18	11	0.0029		0.189542	
		10000	38	24	0.0031		0.202614	

RT Dose	No Virus	899-6 @ MOI 0.1
0	1	1
2	0.694	0.256
5	0.082	0.196

Results - Count plates

RT	Virus	# plated	# counted 1	# counted 2	Counted ave.	P.E.	Survival	Ave survival	
Ø	Ø	10^3	154	104	129	.13	100	100	
		5×10^3	Tm	Tm	—	—	—		
		10^4	Tm	Tm	—	—	—		
2 Gy	Ø	10^3	75	104	90	"	69	69	
		5×10^3	Tm	Tm	—	—	—		
		10^4	Tm	Tm	—	—	—		
5 Gy	Ø	10^3	18	13	16	"	12	8.1	
		5×10^3	20	44	32	"	4.9		
		10^4	71	124	98	"	7.5		
					1.7	.017			
Ø	899-6	10^3	12	7	9.5	"	7.3	11.7	
		5×10^3	68	139	103	"	15.9		
		10^4	Tm	156	156	"	12		
2 Gy	899-6	10^3	3	6	4.5		3.5	4.3	.17
		5×10^3	~~25~~ 13	17	15	"	2.3		
		10^4	25	(160)	25 (92.5)	"	1.9 (7.1)		
5 Gy	899-6	10^3	2	4	3	"	2.3	2.3	
		5×10^3	18	11	14.5	"	2.2		
		10^4	38	24	31	"	2.4		

FINDINGS —

1. R899-6 virus is highly cytotoxic at an MOI of 10^{-1} to T98G cells with a plating efficiency of only 1.5%.
2. Higher levels of TNF are produced in T98G cells than in SQ20B cells = 5 fold more at an MOI of 10^{-1} (250 vs 50).
3. No radiation induction of TNF, i.e. it is produced constitutively.
4. There is no clear synergistic effect between ~~B~~ 5 Gy & 899-6 @ 10^{-1}, although there is possible synergy at 2 Gy.
5. T98G cells are more radiosensitive than SQ20B cells.

SQ 20B

Purpose –
- Quantify cell killing of R3616 at MOI of 10^{-2}, 10^{-1}, 5.
- Is killing additive or synergistic c̄ 5 Gy?
- Quantify the effect of IgG (human) in the incubation media
- ~~Does the IgG~~ Is there a cell density dependent killing, and does IgG eliminate this phenomenon

Design
- Fixed Variables – Cell type: ~~20~~ SQ 20B
  ~~Virus: R3616~~
  Interval ~~from~~ of infection: 2° ~~then~~
  Interval from infection to RT: 16°
  Interval from RT to subcult: 4-6°
- Study Variables – Virus: None, R3616 @ MOI 10^{-2}, 10^{-1}, 5
  human IgG: +, –
  RT: 0 Gy, 5 Gy

SQ-20B

Virus	moi	IgG	RT	
Ø	—	0	0	5.6
"	"	"	"	4.8
Ø	—	+	0	6
Ø	—	0	5	3.4
"	"	"	"	3.7
Ø	—	+	5	4.2
3616	10^{-2}	0	0	4.2
"	"	"	"	inf.
3616	10^{-2}	+	0	5.9
"	"	"	"	4.2
3616	10^{-2}	0	5	2.7
"	"	"	"	2.4
3616	10^{-2}	+	5	2.8
"	"	"	"	4.2
3616	10^{-1}	0	0	5.9
"	"	"	"	5.8
3616	10^{-1}	+	0	3.3
"	"	"	"	3.35
3616	10^{-1}	0	5	2.2
"	"	"	"	1.9
3616	10^{-1}	+	5	1.8
"	"	"	"	1.8
3616	5	0	0	1.8
"	"	"	"	2.5
3616	5	+	0	
"	"	"	"	
3616	5	0	5	
"	"	"	"	
3616	5	+	5	
"	"	"	"	

switch

need 31 plates x 5 ml = 155 ml
∴ resuspend in 165 ml
10^7 cells in 165 ml = 3×10^5/5 ml

Methods –

1) Plate 5×10^5 cells per 60 mm dish

2) Infect c̄ virus in 2 ml 2% FCS

Dilutions: Stock $\xrightarrow{10\,\mu l}$ (40) $\xrightarrow{10}$ (40) $\rightarrow$ (40)

6×10^6 PFU/ml, 6×10^5, 6×10^4, 6×10^3

Count dish = 2×10^6 cells

- MOI 10^{-2} = 2×10^4 PFU / 6×10^3 PFU/µl = 3.3 µl of 6×10^3 dilution
- MOI 10^{-1} = 2×10^5 cells / 6×10^4 PFU/µl = 3.3 µl of 6×10^4 dilution
- MOI 5 = 1×10^7 PFU / 6×10^5 PFU/µl = 16.7 µl of 6×10^5 dilution

3) Incubate 2° then ~~add~~ aspirate media &

add: 4 ml of 2% FCS (⊖ IgG) or

4 ml of 2% FCS + human γglob (1µl/ml) (stock = 165mg/10…

4) Irradiate c̄ 0 or 5 Gy

5) Subculture & count each dish c̄ hemacytometer

6) Make dilutions 10^6, 10^5, 10^4, 10^3 for each dish

7) Plate 3 dilutions for each original dish = 5×10^2, 10^3, 5×10^3

8) Incubate for 10d–14d

1	2	3	4	5	6	7	8	9
VIRUS	MOI	IgG	RT	# plated	# counted			
3616	10^{-1}	−	0	500	96			
"	"	+	0	500	101			
"	"	+	0	500	251			
"	"	−	0	10^3	TM			
"	"	+	0	10^3	197			
"	"	+	0	10^3	TM			
~~"~~	~~"~~	~~−~~	~~0~~	~~5×10^3~~				
~~"~~	~~"~~	~~+~~	~~0~~	~~5×10^3~~				
~~"~~	~~"~~	~~+~~	~~0~~	~~5×10^3~~				
3616	10^{-2}	−	0	500	214			
"	"	"	0	500	130			
"	"	+	0	500	177			
"	"	"	0	500	205			
"	"	−	0	10^3	231			
"	"	"	0	5×10^3	TM			
3616	10^{-2}	−	5	500	49			
"	"	"	5	500	43			
"	"	+	5	500	25			
"	"	"	5	500	48			
"	"	−	5	10^3	90			
"	"	"	5	10^3	68			
"	"	+	5	10^3	47			
"	"	"	5	10^3	49			
"	"	−	5	5×10^3	Tm			
"	"	"	5	5×10^3	Tm			
"	"	+	5	5×10^3	205			
"	"	"	5	5×10^3	287			

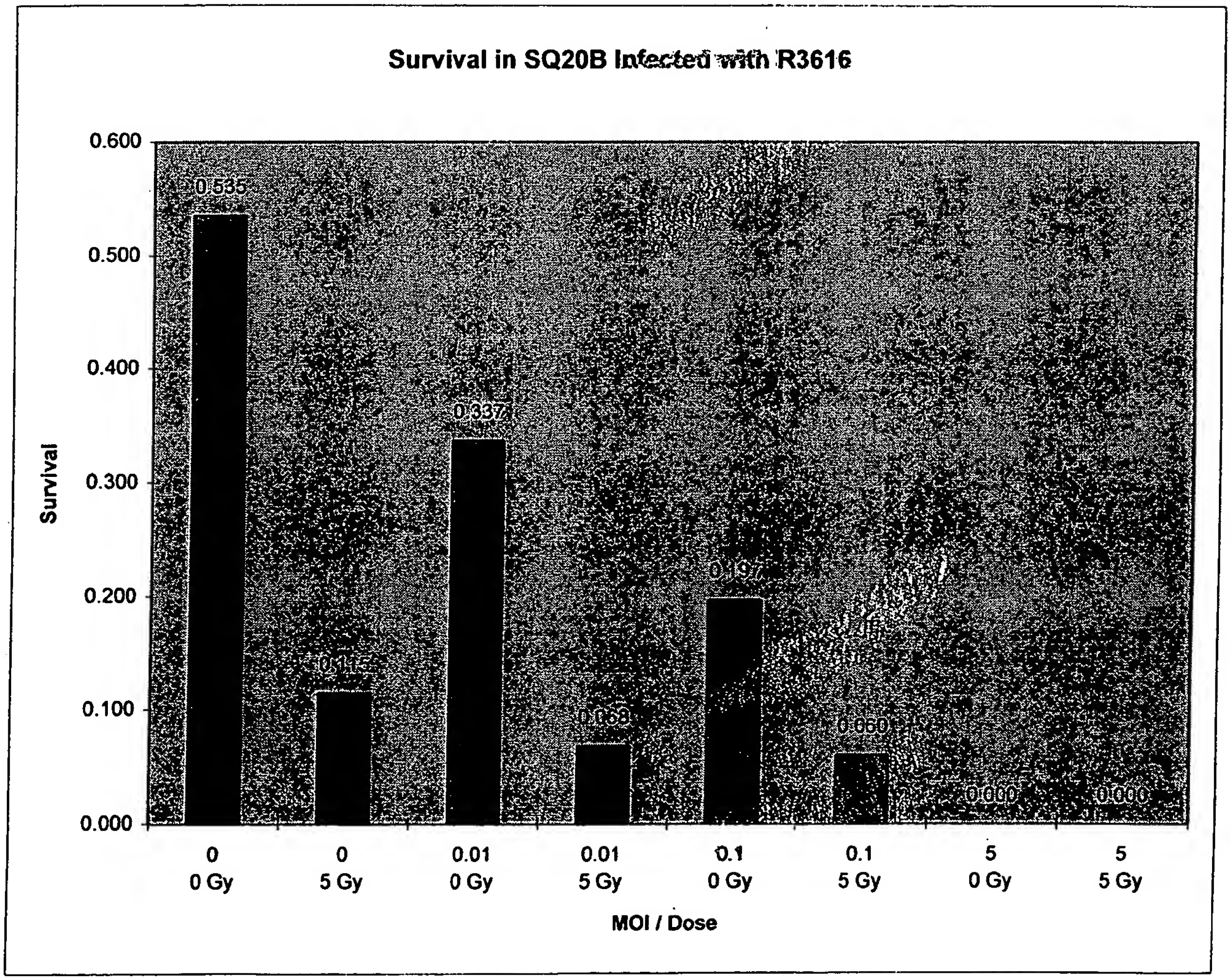
SQ-2013

Virus	moi	IgG	RT	# plated	# counted
0	–	–	0	500	323
		–	0	500	203
		+	0	500	277
				$10^3/5\times10^3$	TM
0	–	–	5	500	36
"	"	"	"	"	72
0	–	–	5	10^3	89
"	"	"	"	"	130
0	"	+	5	5×10^3	TM
0	–	+	5	500	56
"	–	+	5	10^3	140
"	"	"	"	5×10^3	TM
3616	10^{-1}	–	5	500	51
"	"	"	"	500	25
"	"	+	"	500	31
"	"	"	"	500	38
"	"	–	"	10^3	68
"	"	"	"	10^3	55
"	"	+	"	10^3	59
"	"	"	"	10^3	77
"	"	–	"	5×10^3	215
"	"	"	"	5×10^3	185
"	"	+	"	5×10^3	246
"	"	"	"	5×10^3	225

SQ 20B: R3616 BY MOI AND+/- IgG								
Virus	MOI	IgG	RT	# plated	# counte	Crude SV	PE	Rel. SV
0	-	-	0	500	323	0.646	0.535333	100
0	-	-	0	500	203	0.406		
0	-	+	0	500	277	0.554		
0	-	-	5	500	36	0.072	0.533	0.214822
0	-	-	5	500	72	0.144		
0	-	+	5	500	56	0.112		
0	-	+	5	1000	140	0.14		
0	-	-	5	1000	89	0.089		
0	-	-	5	1000	130	0.13		
R3616	0.01	-	0	500	214	0.428	0.3366	100
R3616	0.01	-	0	500	130	0.26		
R3616	0.01	+	0	500	177	0.354		
R3616	0.01	+	0	500	205	0.41		
R3616	0.01	-	0	1000	231	0.231		
R3616	0.01	-	5	500	49	0.098	0.337	0.202733
R3616	0.01	-	5	500	43	0.086		
R3616	0.01	+	5	500	25	0.05		
R3616	0.01	+	5	500	48	0.096		
R3616	0.01	-	5	1000	90	0.09		
R3616	0.01	-	5	1000	68	0.068		
R3616	0.01	+	5	1000	47	0.047		
R3616	0.01	+	5	1000	49	0.049		
R3616	0.01	+	5	5000	205	0.041		
R3616	0.01	+	5	5000	287	0.0574		
R3616	0.1	-	0	500	96	0.192	0.197	
R3616	0.1	+	0	500	101	0.202		
R3616	0.1	+	0	500	[251]	-		
R3616	0.1	-	0	1000	TM	-		
R3616	0.1	+	0	1000	197	0.197		
R3616	0.1	+	0	1000	TM	-		
R3616	0.1	-	5	500	51	0.102	0.197	0.305922
R3616	0.1	-	5	500	25	0.05		
R3616	0.1	+	5	500	31	0.062		
R3616	0.1	+	5	500	38	0.076		
R3616	0.1	-	5	1000	68	0.068		
R3616	0.1	-	5	1000	55	0.055		
R3616	0.1	+	5	1000	59	0.059		
R3616	0.1	+	5	1000	77	0.077		
R3616	0.1	-	5	5000	215	0.043		
R3616	0.1	-	5	5000	185	0.037		
R3616	0.1	+	5	5000	246	0.0492		
R3616	0.1	+	5	5000	225	0.045		
R3616	5	+	0	10000	0	0	0	0
R3616	5	+	5	10000	0	0		

SQ 20B: R3616 BY MOI AND+/- IgG

Dose	MOI	Survival
0 Gy	0	0.535
5 Gy	0	0.115
0 Gy	0.01	0.337
5 Gy	0.01	0.068
0 Gy	0.1	0.197
5 Gy	0.1	0.060
0 Gy	5	0.000
5 Gy	5	0.000



FINDINGS

In SQ-20B:

- There is 63% & 37% survival @ MOI of 10^{-2} & 10^{-1} (corrected for plating efficiency)
- 5 Gy radiation results in 21% survival $\bar{s}$ virus (corrected for plating efficiency)
- Cell killing is <u>additive</u> between virus and radiation at the MOI & dose studied.
- An MOI of 5 results in complete cell killing
- Human γ-globulin in the ~~serum~~ media did not have an effect on survival
- No cell density dependence was observed

V 87 cells

EFFICIENCY LINE® 22-206

Purpose -

- Compare cytotoxicity of R3616 & R899-6 in V-87 cells (human glioblastoma).
- Determine radiosensitivity of V-87 cells to 5 Gy
- ~~Are~~ Is viral cytotoxicity additive or synergistic with radiation toxicity?
- Quantitate TNF production in R899-6 cells. Does RT (5 Gy) increase TNF production?

Design -

- Fixed variables - cell type: V-87 (human glioblastoma)

Study variables - virus: None, R3616, R899-6

MOI: 10^{-3}, 10^{-1}

RT: 0, 5 Gy

U-87 cells

Virus	MOI	RT	TNF assay
Ø	—	0	P only
"	—	0	no
"	—	5	P only
"	—	5	no
3616	10^{-1}	0	no
"	"	"	no
3616	10^{-1}	5	no
"	"	"	no
3616	10^{-3}	0	P only
"	"	"	no
3616	10^{-3}	5	P only
"	"	"	no
899-6	10^{-1}	0	Y
"	"	"	Y
899-6	10^{-1}	5	Y
"	"	"	Y
899-6	10^{-3}	0	Y
"	"	"	Y
899-6	10^{-3}	5	Y
"	"	"	Y

Extra plate to count

Need 6 plates = Ø virus
3616 10^{-1}
3616 10^{-3}
899-6 10^{-1}
899-6 10^{-1}
count

20×5

∴ Need 21 plates × 5 ml = 105 ml
resuspend cells in 110 ml

Plate 10^5, ∴ 2.2×10^6 cells in 110 ml

I didn't do this. No TNF assays done

U87 cells

Methods

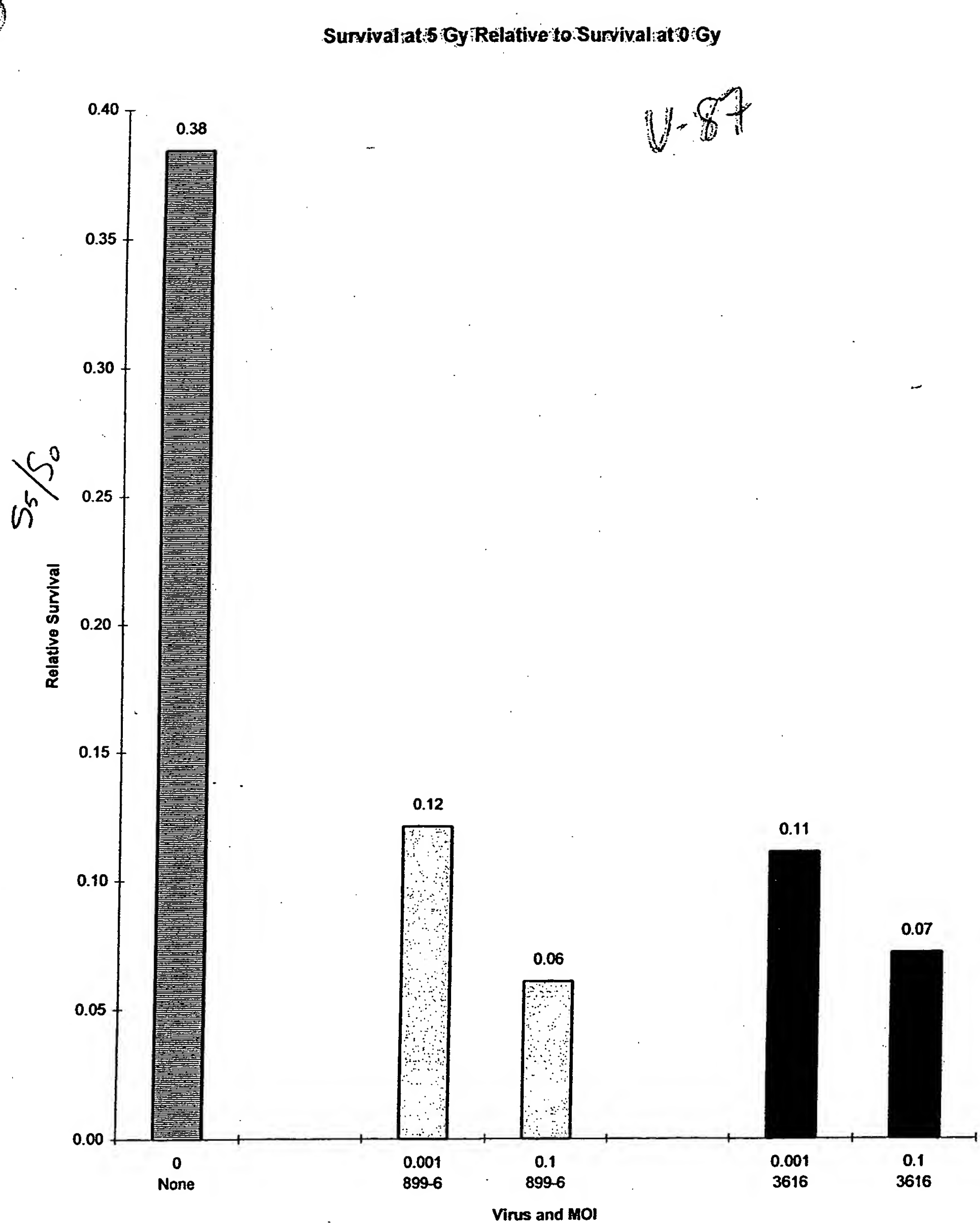
1. Plate 10^5 cells per 60mm plate, Need 6 plate
2. When >90% confluent, infect c̄ virus. Count cells in extra plate to determine PFU's.
   Make dilutions of virus = 6×10^6 PFU/µl (stock) → 6×10^5 → 6×10^4 → 6x
   Count dish = 2.65×10^6
   - moi 10^{-1} = $\frac{2.65 \times 10^5 \text{ PFU}}{6 \times 10^4 \text{ PFU/µl}}$ = 4.4 µl
   - moi 10^{-3} = $\frac{2.65 \times 10^3 \text{ PFU}}{6 \times 10^2 \text{ PFU/µl}}$ = 4.4 µl
3. Incubate 2° in 2% FCS, then subculture dish & make dilutions = 10^6, 10^5, 10^4, 10^3 cells/ml, 10^2
4. Plate dilutions:
   Ø virus, Ø RT = 200, 500, 1000
   Ø virus, 5 Gy = 5×10^3, 10^4, [illegible] $\times 10^4$
   virus, Ø RT = ~~5×10^2~~, 10^3, 5×10^3, 10^4
   virus, 5 Gy = 5×10^3, 10^4, 5×10^4, ~~10^5~~
   Media = 10% FCS + P/S + 1% NaPyr + 1% Ess a.a. in MEM ± 8-96b 1µl/ml
5. Wait 4°, then irradiate
6. Incubate
7. Stain & Count

U-87

Note: poorly defined plaques, slow growing

Results -

Virus	MOI	RT	# plated	# counted	Crude SV	PE	Rel/SV
∅	–	0	200	0/0			
			500	01			
			1000	6			
∅	–	5	5000	1/2			
			10^4	0/12			
			2×10^4	42/45			
899-6	10^{-3}	0	1000	21/6			
			5000	61			
			10^4	?/?			
899-6	10^{-3}	5	5000	1/1			
			10^4	0/7			
			5×10^4	?/106			
899-6	10^{-1}	0	1000	35/6			
			5000	125/69			
			10^4	?/?			
899-6	10^{-1}	5	5000	1/0			
			10^4	6/8			
			5×10^4	138/148			
3616	10^{-3}	0	1000	3/0			
			5000	68/43			
			10^4	?/?			
3616	10^{-3}	5	5000	0/1			
			10^4	12/14			
			5×10^4	?/?			
3616	10^{-1}	0	1000	14/8			
			5000	106/?			
			10^4	?/?			
3616	10^{-1}	5	5000	1/0			
			10^4	4/22			
			5×10^4	?/104(?)			



Survival at 5 Gy Relative to Survival at 0 Gy
V-87
S_5/S_0
Relative Survival
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.38
0.12
0.06
0.11
0.07
0
None
0.001
899-6
0.1
899-6
0.001
3616
0.1
3616
Virus and MOI

U-87

RESULTS:

Virus	MOI	RT	# plated	# counted		Crude SV	Ave SV	PE	Rel SV	
None	-	0	200	0	0	0	0.002667	0.002667	1	
None	-	0	500	1		0.002				
None	-	0	1000	6		0.006				
None	-	5	5000	1	2	0.0003	0.001025	0.002667	0.384375	
None	-	5	10,000	0	12	0.0006				
None	-	5	20,000	42	45	0.002175				1/2
899-6	0.001	0	1000	21	6	0.0135	0.00735	0.00735	1	
899-6	0.001	0	5000	6		0.0012				
899-6	0.001	0	10,000	tm	tm					
899-6	0.001	5	5000	1	1	0.0002	0.00089	0.00735	0.121088	
899-6	0.001	5	10,000	0	7	0.00035				
899-6	0.001	5	50,000	106	tm	0.00212				
899-6	0.1	0	1000	35	6	0.0205	0.01995	0.01995	1	
899-6	0.1	0	5000	125	69	0.0194				
899-6	0.1	0	10,000	tm	tm					
899-6	0.1	5	5000	0	1	0.0001	0.00122	0.01995	0.061153	
899-6	0.1	5	10,000	6	8	0.0007				
899-6	0.1	5	50,000	138	148	0.00286				
3616	0.001	0	1000	3	0	0.0015	0.0063	0.0063	1	?
3616	0.001	0	5000	68	43	0.0111				
3616	0.001	0	10,000	tm	tm					
3616	0.001	5	5000	0	1	0.0001	0.0007	0.0063	0.111111	
3616	0.001	5	10,000	12	14	0.0013				
3616	0.001	5	50,000	tm	tm					
3616	0.1	0	1000	14	8	0.011	0.0161	0.0161	1	?
3616	0.1	0	5000	106	tm	0.0212				
3616	0.1	0	10,000	tm	tm					
3616	0.1	5	5000	1	0	0.0001	0.00116	0.0161	0.07205	
3616	0.1	5	10,000	4	22	0.0013				
3616	0.1	5	50,000	104	tm	0.00208				

variable

time I → S

I → X

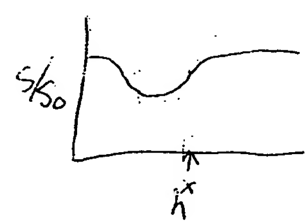
S → X

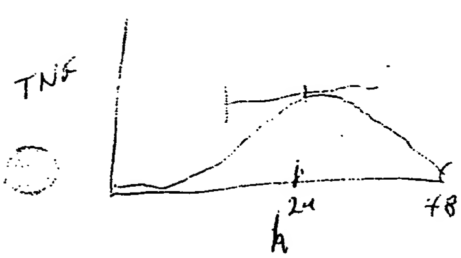
schedule

I → X → S

PLDR

S → I → X





I → ELISA

SQ20B

EFFICIENCY LINE® 22-206

Purpose

- Determine viral cytotoxicity of R3616 @ moi of 10^{-} & 1 in SQ20B cells
- Is viral cell killing additive or synergistic with 9 Gy RT?

Design

- Fixed variables - Cell type: SQ20B
~~Virus~~
Interval infection → subculture: 2 hour
Interval subculture → RT : 4 hours
Human γ globulin added to plates p̄ subculture

- Study variables - Virus : R3616 @ moi 0, 10^{-1}, 1
RT : 0, 9 Gy

TITERS

Cell	Virus	Dilution	Plaque #	Titer
SQ 20B	3616	10^7	0	?
		"	0	
		10^8	0	
		"	0	
SQ 20B	899-6	10^7	0	?
		"	0	
		10^8	0	
		"	0	
V-87	3616	10^7	~~39~~ ~~18~~ 8	6×10^7
		"	~~42~~ 3	
		10^8	~~8~~ 0	
		"	~~7~~ 0	
V-87	899-6	10^7	~~33~~ 13	1.1×10^8
		"	~~43~~ 8	
		10^8	~~8~~ 0	
		"	~~7~~ 0	
T-986	3616	10^7	14	1.2×10^8
		"	9	
		10^8	0	
		"	0	
T986	899-6	10^7	12	4.1×10^8
		"	39	
		10^8	3	8×10^8
		"	7	

none seen, but cells overgrown. Try titering @ 80% confluence

small plaques

nice plaques

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 18773

ARC USE ONLY

P.O. #
ORDER DATE
REF. #
CONTACT
EST AMT
SCHED DEL
SPECIAL ROUTING
NON COM VENDOR
FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Greg Sibley, MD DATE: ______

REQUESTORS PHONE NUMBER: 312-702-0284

AUTHORIZED SIGNATURE: [signature]

FAS ACCOUNT: 6-95150-5100 P.I.: Hallahan

VENDOR: Jackson Labs PROTOCOL: 58671

REQUESTED DELIVERY DATE: ASAP PHONE: 2-6829

SPECIES: mouse QUANTITY: 24

STRAIN: nu/J SEX: M F EITHER

WEIGHT/AGE: 5-7 wks ALTERNATE WEIGHT/AGE: ______
(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☑ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS: ______

HOUSE AT: ___ CARLSON ___ WYLER X CLSC ___ FMI ___ OTHER

T986

PURPOSE -

- Establish dose response survival curves with T986 cell line. ~~Doses~~
- ~~Establish dose response survival curve with T986 cell line + EN R3616 @ MOI = 10^{-1}.~~

DESIGN -

- Fixed variables - cell type T986 (human glioblastoma)
  - ~~intervals - infect x 2° → subculture~~
    ~~wait 4° → irradiate~~
- Study variables - virus: None, ~~R3616 @ MOI = 10^{-1}~~
  RT dose: 0, 2, 3, 5, 7, 9, 11 Gy

METHODS

① Plate 7986 cells on 60 mm dish & grow to ~90% confluence
Need 5 plates. (Plate 10^5/dish & 5×10^4/dish = 10 dishes & choose best set)

② Count 1 plate & infect ~~others~~ 2 plates c̄ R3616 @ MOI = 10^{-1} c̄ 199V × 2°.
Count = 1.6×10^6 cells, MOI 10^{-1} = $\frac{1.6\times10^5}{6\times10^4 \text{ PFU}/\mu l \text{ virus dilution}}$ = ~~2.7 µl~~
* I infected @ MOI = 3.75 by mistake

③ After 2° infection subculture to 100 mm plates (in duplicate)

RT	Virus	# plated		
0	–	200	500	~~1000~~
2	–	~~200~~	500	1000
3	–	~~500~~	1000	2000
5	–	2000	5000	~~10^4~~
7	–	~~10^4~~	5000	10^4
9	–	10^4	10^5	
~~#~~				
~~0~~	+	2000	5000	
~~2~~	+	5000	10^4	
~~3~~	+	5000	2×10^4	
~~5~~	+	10^4	5×10^4	
~~7~~	+	5×10^4	10^5	
~~9~~	+	10^5	~~2×10~~	
~~#~~	~~#~~			

∴ can't use

T986

RESULTS

Virus	RT Dose	# Plated	# Counted		ave Surv.	P.E.	rel Surv.
Ø	0	200	1	0	—		
		500	0	—			
	2	500	0	4	5×10^{-3}		
		1000	9	2			
	3	2000	1	8	2.2×10^{-3}		
		1000	3	1			
	5	2000	1	6	2.1×10^{-3}		
		5000	3	—			
		10^4	29	—			
	7	5000	2	4	7.7×10^{-4}		
		10^4	8	9			
	9	10^4	3	7	5×10^{-4}		
		10^5	Tm	Tm			

Conclusions

① Poor plating efficiency. Can't draw any conclusions. Repeat.

Titer ~~cells~~ viruses in 4 cell lines

METHODS -

1. Plate cells in T-25's on Friday for use Mon.
   - Vero cells plate 10^5/flask
   - T98G cells plate 2×10^5/flask
   - 5Q 20B cells plate 3×10^5/flask
   - U87 cells plated earlier this week

	1	2	3	4	5	6	7	8	9
	VERO CELLS								
		D_1	D_2	D_3					
T-25	10^5	30%	40%	sub conf 85-90 -100% ~~conf~~					
T-25	2×10^5	50%	80%						
T-25	5×10^5	80-90%	-100%	confl +					
T-150	10^5	—	—						
	T986	D_1	D_2	D_3					
T-25	5×10^4	–	30%						
T-25	10^5	–	50%						
T-25	2×10^5	30%	70%						
	V-87								
		D_1							
	10^5								

In-vitro - SQ-20B

1.) Titer virus in SQ-20B

2.) Generate Survival curve for R3616 @ 0, 3, 5, 7, 9, 11 Gy - MOI = 0

- U87

1.) Titer virus in U-87

2.) Generate Survival curve for R3616 (MOI = 10^{-3}) @ 0, 2, 3, 5, 7 Gy
R3616 (MOI = 10^{-1})
R899-6 (MOI = 10^{-3})
R899-6 (MOI = 10^{-1})

- T98G

1.) Titer virus in U-87

2.) Generate survival curves as above for U87

TNF

1.) Measure in U-87 & T98G infected c̄ R3616 to r/o cellular ~~viral~~ TNF production in response to virus

2.) Quantify

TITERS

Cell	Virus	Dilution	Plaque	ct	Titer	
Vero	3616	10^6	TM	TM		
		10^7	230	bad flask		
		10^8	23	24	2.3×10^9	
Vero	899-6	10^6				
		10^7	104	117	1.2×10^9	
		10^8	15	12		
T98G	3616	10^5				
		10^6	35*			
		10^7	12			
T98G	899-6	10^5				
		10^6	171			
		10^7	19			
U 87	3616	10^5				
		10^6				
		10^7				
U 87	899-6	10^5				
		10^6				
		10^7				
SQ 20B	3616	10^4				10^2
		10^5				10^3
		10^6				10^4
SQ 20B	899-6	10^4				10^{-5}
		10^5				10^5
		10^6				10^6

U-87

PURPOSE -

- Establish dose response survival curve with U87 cell line
- Establish dose response survival curve with U87 cell line + R3616 @ MOI = 10^{-1}

DESIGN -

- Fixed variables - cell type U87 (human glioma)
  - intervals = infect × 2° → subcult → 4° → XRT
- Study variables - virus: None, R3616 @ MOI = 10^{-1}
  - RT dose: 0, 2, 3, 5, 7, 9 Gy

U-87

METHODS

① Plate U87 cells on 60 mm dish & grow to ~80% confluence. Need 5 plates. (plate 5×10^5/plate)

② Count 1 plate & infect 2 plates @ MOI 10^{-1} (R3616) for 2°

1.2×10^6 cells $\times 10^{-1}$ (MOI) = 1.2×10^5 PFU / 6×10^5 PFU/ml dil. = .2 ml + .8 ml

③ After 2° infection, subculture to 100mm plates (in duplicate

RT	Virus	# plated		
0	−	500	1000	5000
2	−	1000	5000	10^4
3	−	5000 ~~10^4~~	10^4	2×10^4
5	−	10^4	2×10^4	
7	−	~~10^4~~	5×10^4	10^5
9	−	5×10^4	10^5	
0	+	1000	2000	5000
2	+	5000	10^4	
3	+	10^4	2×10^4	
5	+	10^4	2×10^4	5×10^4
7	+	5×10^4	10^5	
9	+	5×10^4	10^5	

④ Stain & count

U-87

RESULTS

R3616 moi	RT Dose	# Plated	# counted		ave survival	P.E.	rel. survival
0	0	500	0	0	5×10^{-4}	5×10^{-4}	1
		1000	0	0			
		5000	2	3			
	2	1000	0	0	2.7×10^{-4}		.53
		5000	0	1			
		10^4	4	3			
	3	5000	0	0	1.55×10^{-3}		3.1
		10^4	2	6			
		2×10^4	47	38			
	5	2×10^4	13	5	3.2×10^{-4}		.63
		10^4	1	0			
	7	5×10^4	5	5	6×10^{-4}		1.2
		10^5	104	67			
	9	5×10^4	1	2	4.1×10^{-4}		.83
		10^5	69	52			

V-87

Results (cont.)

R3616 Mol	RT Dose	# Plated	# counted		ave. surv.	P.E.	rel surv.
0.1	0	1000	0	0	3×10^{-4}	3×10^{-4}	1
		2000	0	0			
		5000	2	1			
	2	5000	0	0	3×10^{-4}		1
		10^4	3	3			
	3	10^4	3	0	1.1×10^{-3}		3.7
		2×10^4	41	—			
	5	10^4	2	0	1.4×10^{-3}		4.9
		2×10^4	20	23			
		5×10^4	105	85			
	7	5×10^4	36	16	8.7×10^{-4}		2.9
		10^5	92	116			
	9	5×10^4	20	10	2.6×10^{-4}		.88
		10^5	4	45			

?

U-87

CONCLUSIONS

① U87 is a fairly radioresistent cell line (surviving cells @ 9 Gy)

② Does not appear to be a major impact of R3616 on dose response curve.

③ Plating efficiency extremely low (= 5 x 10^{-4}) Cannot draw any quantitative conclusions

④ Suit estimates SF_2 (in vitro) at 0.5.

⑤ Repeat using conditioned media

EFFICIENCY LINE® 22-206

Purpose -

- Establish dose response survival curve with U87 cell line & 839-6 virus @ 10^{-1} & 10^{-3}

Design -

- Fixed variables - cell type U87
  - intervals = infect ×2° → subcult → 4° → XRT

- Study variables - virus MOI = 10^{-1} & 10^{-3} 839-6
  - RT dose : 0, 2, 3, 5, 7, 9 Gy

METHODS –

① Plate U87 cells on 3 60mm dishes = 5×10^5

② ~~Count~~ Grow to subconfluence, Count 1 dish to calculate MOI. Count = 3×10^6 cells

New 888-6 titer = 1.2×10^9 PFU/ml

For MOI 10^{-1}: 3×10^5 PFU / 1.2×10^6 PFU/ml dil = ~~[illegible]~~ .25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

For MOI 10^{-3}: 3×10^3 PFU / 1.2×10^4 PFU/ml dil = ~~250 ml~~ .25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

③ Infect x 2° then subcult to 100mm plates (in dupl)

RT	Virus	# plated		
0	10^{-1}	1000	2000	5000
2	10^{-1}	5000	10^4	
3	10^{-1}	10^4	2×10^4	
5	10^{-1}	10^4	2×10^4	
7	10^{-1}	5×10^4	10^5	
9	10^{-1}	5×10^4	10^5	
0	10^{-3}	1000	2000	5000
2	10^{-3}	5000	10^4	
3	10^{-3}	10^4	2×10^4	
5	10^{-3}	10^4	2×10^4	
7	10^{-3}	5×10^4	10^5	
9	10^{-3}	5×10^4	10^5	
0	Ø	500	1000	5000

④ Stain & count (perfect size to count)

V-87

RESULTS -

899-6 LMB1	RT	# Plated	# Counted		ave surv.	P.E.	rel surv.
10^{-1}	0	1000	0	—	—	.008	1
		2000	1 —	1 —	—		
		5000	23	57	.008		
	2	5000	11	3	.0045		.56
		10^4	74	47			
	3	10^4	16	10	.0024		.29
		2×10^4	68	TM			
	5	10^4	0	5	.002		.25
		2×10^4	55	24			
	7	5×10^4	61	81	.0014		.18
		10^5	TM	TM			
	9	5×10^4	17	325	.0004		.05
		10^5	84?	TM			
10^{-3}	0	1000	2 —	0 —	.0081 ~~.0067~~	.0081 ~~.0067~~	1
		2000	7 —	9 —			
		5000	43	38			
	2	5000	4 —	3 —	.0058		.71
		10^4	60	55			
	3	10^4	37	50	.0044		.54
		2×10^4	TM	TM			
	5	10^4	10	21	.0026		.32
		2×10^4	63	62			
	7	5×10^4	98	97	.002		.24
		10^5	TM	TM			
	9	5×10^4	34	39	.0007		.09
		10^5	TM	TM			

EFFICIENCY LINE® 22-206

AMPAD

	1	2	3	4	5	6	7	8	9	
1										
2										
3										
4										
5										
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30										
31										

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 22004

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Dr. Greg Sibley DATE: ______

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: [signature]

FAS ACCOUNT: 6-95150-5100 | P.I.: Hallahan

VENDOR: FCRI | PROTOCOL: 58671

REQUESTED DELIVERY DATE: ______ | PHONE: 2-6819

SPECIES: mouse | QUANTITY: 48

STRAIN: Athymic Nude | SEX: M F EITHER

WEIGHT/AGE: 5-6wks ALTERNATE WEIGHT/AGE: ______
(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☑ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: ______

HOUSE AT: ____ CARLSON ____ WYLER X CLSC ____ FMI ____ OTHER

U87 - TNF

EFFICIENCY LINE® 22-206

Purpose - Quantify TNF production in U87 cell line with 899-6 virus +/- 5 Gy RT.
Use 3616 virus as control.

Design -
- Fixed variables - cell type U87
- intervals = infect x 2 hrs → RT
- aliquot media @ 0, 2, 4, 6 hrs then subculture & save 10^6 cells

- Study variables - virus: None, 3616, 899-6 @ MOI = 10
RT: 0 Gy, 5 Gy

Conclusion - 10^5 PFU too little & slow TNF production c̄ 899-6

METHODS –

(1) Plate U87 cells in 60 mm dishes.
Grow to subconfluent. Need ~~20~~ 15 dishes

(2) ~~Count 1 dish to determine # PFU needed for MOI of 10.~~ No. Infect c̄ 10^5 PFU (use this also for other cell lines to compare TNF production i.e. TNF production should be dependent only on PFU & not on cell number (MOI not important)).

899-6 titer = 1.2×10^9 PFU/ml : $\frac{10^5 \text{ PFU}}{1.2 \times 10^5 \text{ PFU/ml}}$ = 0.83 ml of 1.2×10^5 dil (+ .17 media)

3616 titer = 2.3×10^9 PFU/m : $\frac{10^5}{2.3 \times 10^5 \text{ PFU/ml}}$ = 0.43 ml " 2.3×10^5 " (+ .57 media)

(3) Infect cells x 2° in 1 ml 199V media.
~~Add~~

(4) Remove innoculum & add 6 ml of 10% FCS media (U87 media) + IgG.

(5) Irradiate c̄ 5 Gy

(6) Aliquot 1 ml media @ 0, 2, 4, 6° & subculture in 5 ml ~~@ 10^6 cells/ml~~ → save ~~as~~ 1 ml as "pellet".

(7) Centrifuge aliquots before using for TNF ELISA assay

(8) To calculate total TNF:

899-6 = ([0°] × 1 ml) + ([2°] × 1 ml) + ([4°] × 1 ml) + ([6°] × 3 ml) + ([6° P] × 3 ...

3616 = ([6°] × 6 ml) + ([6° P] × 5 ml)

Ø = ([6°] × 6 ml) + ([6° P] × 3 ml)

U-87-R899-6

Purpose - Generate cell survival curve for U-87 cells with different MOI's of 899-6 virus.

Design - Fixed variables - cell type U87
- intervals = infect x 2°
- virus = 899-6

Study variables = MOI = 2, 1, .5, 10^{-1}, ~~5×10^{-2}~~, 10^{-2}, ~~5×10^{-3}~~, 10^{-3}, 10^{-4}

V-87

METHODS -

① Plate 5×10^5 U87 cells / 60 mm plate, grow to sub-confluence.

② Subculture & count a plate = 2.4×10^6 cells

titer of 89-6 = 1.2×10^9 PFU/ml → dilutions

moi 2 = $4.8 \times 10^6 / 1.2 \times 10^7$ = .4 ml

moi 1 = $2.4 \times 10^6 / 1.2 \times 10^7$ = .2 "

moi 0.5 = $1.2 \times 10^6 / 1.2 \times 10^7$ = .1 "

moi 0.1 = $2.4 \times 10^5 / 1.2 \times 10^6$ = .2 "

moi 5×10^{-2} = $1.2 \times 10^5 / 1.2 \times 10^6$ = .1 "

moi 10^{-2} = $2.4 \times 10^4 / 1.2 \times 10^5$ = .2 "

moi 5×10^{-3} = $1.2 \times 10^4 / 1.2 \times 10^5$ = .1 "

moi 10^{-3} = $2.4 \times 10^3 / 1.2 \times 10^4$ = 0.2 "

moi 10^{-4} = $2.4 \times 10^2 / 1.2 \times 10^3$ = .2 "

③ Infect x 2 hrs

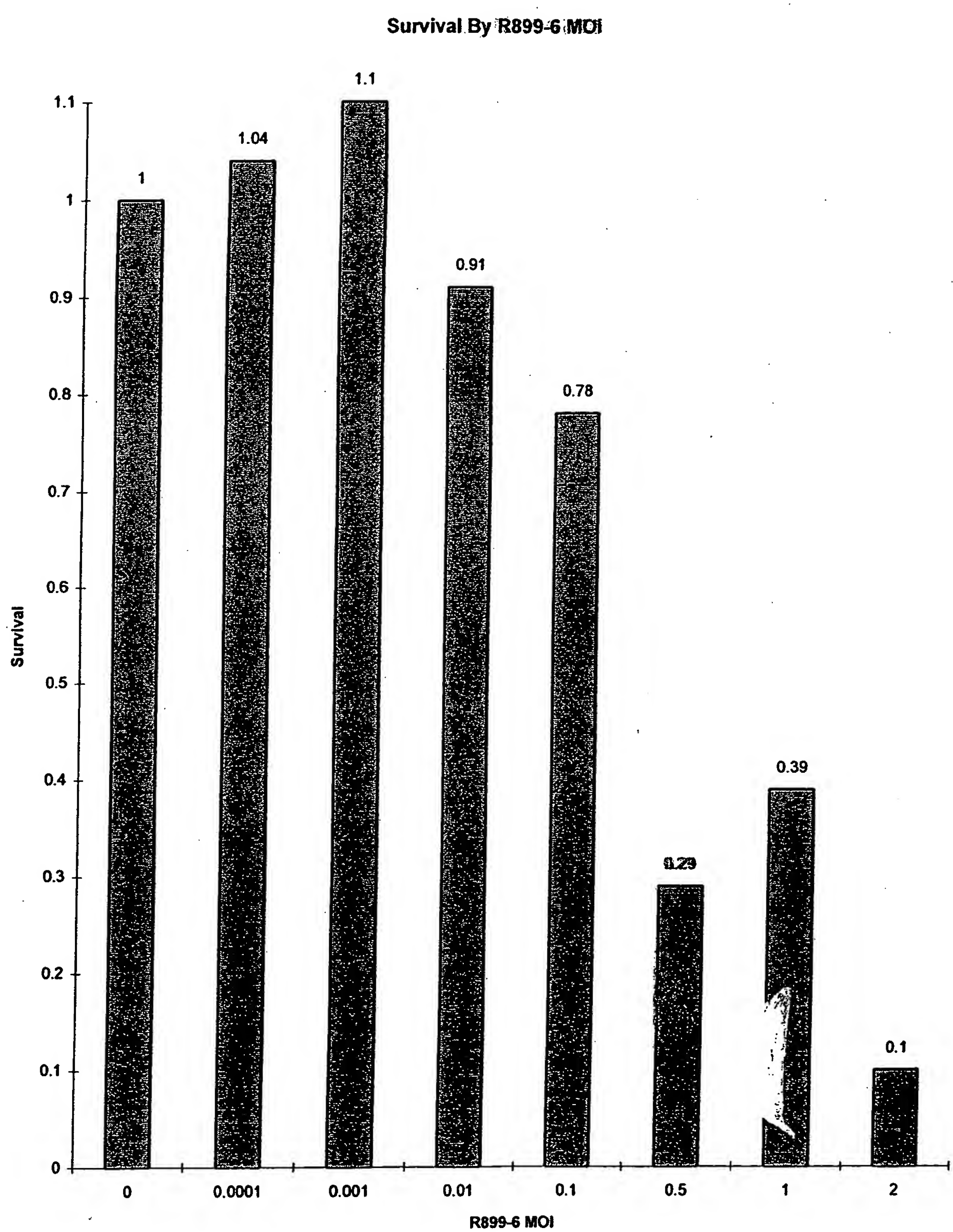
④ Subculture to 100 mm plates c̄ 10% FCS media ⊕ IgG

⑤ Stain & count p̄ ~ 3 wks (too soon = plaques too sm

V-87

RESULTS

899-6 mol	# Plated	# counted		ave. survival	P.E.	rel. survival
0	500	12	—	.021	.021	1
	1000	25	—			
	2000	35	45			
10^{-4}	1000	24	30	.022	"	1.04
	2000	35	44			
	5000	Tm	Tm		'	
10^{-3}	2000	70	62	.023	"	1.10
	5000	104	91			
	10^4	Tm				
10^{-2}	2000	24	41	.019	"	.91
	5000	95	112			
	10^4	Tm	Tm			
10^{-1}	2000	32	34	.016	"	.78
	5000	Tm	Tm			
	10^4	Tm				
0.5	5000	34	28	.0062		.29
	10^4	33	Tm			
	10^5	Tm				
1	10^4	46	36	.0082		.39
	10^5	Tm	Tm			
	10^6	Tm				
2	10^5	>150 Tm	>150 Tm	>.0015		>.07
	10^6	Tm				


Survival By R899-6 MOI
Survival
R899-6 MOI
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
1.04
1.1
0.91
0.78
0.29
0.39
0.1
0
0.0001
0.001
0.01
0.1
0.5
1
2

U-87

AMPAD EFFICIENCY LINE® 22-206

Conclusions –

① R899-6 doesn't appear to have an appreciable effect until moi ~ 0.1

② ? TCD_{50} ~ moi = 0.5

③ Repeat using moi's of 0, 10^{-2}, 10^{-1}, 0.5, 1, 2, 3, 5.

④ Plating efficiency still very low (2%), try using conditioned media (50/50)

TNF ELISA — Adeno-TNF Expression (#53) 2.2x10^8 pfu/tumor

Adeno Egr TNF + single 5 Gy fractions

	1	2	3	4	5	6	7	8	9	10
A	0		1394 R		1406 R		1410 R		1394 L	
B	15.7		1396 R		1403 R		1415 R		1396 L	
C	31.3		1434 R		1404 R		1412 R		1434 L	
D	62.5		1397 R		1405 R		1413 R		1397 L	
E	125		1398 R		1402 R		1414 R		1398 L	
F	250		1399 R		1407 R		1411 R		1399 L	
G	500		1400 R		1408 R		1416 R		1400 L	
H	1000		1401 R		1409 R		RIPA Blk		1401 L	

Adeno Ø xrt (columns 9–10 header: Ø xrt)

Adeno-βgal control 5 Gy 2x10^8 pfu/tumor Ø xrt

	11	12	13	14	15	16	17	18	19	20
A	1406 L		1410 L		1421		1417		1433 R	
B	1403 L		1415 L		1422		1418		1433 L untreat tumor	
C	1404 L		1412 L		1423		1420		1419	
D	1405 L		1413 L		1425		1424		media blk - Gne	
E	1402 L		1414 L		1436 R		1427		1 899-6 0°	
F	1407 L		1411 L		1436 L untreated tumor		1429		2 " 0 Gy	
G	1408 L		1416 L		1428		1426		3 899-6 2°	
H	1409 L		RIPA Blk		1435		1430		4 " 0 Gy	

	21	22	23	24	25	26	27	28
A	5 899-6 4°		13 ~~None~~ 899-6 2°		21 None 6°		29 3616 6°	
B	6 " 0 Gy		14 " 5 Gy		22 " 0 Gy		30 " 0 Gy	
C	7 899-6 6°		15 899-6 4°		23 None P		31 3616 P	
D	8 " 0 Gy		16 " 5 Gy		24 " 0 Gy		32 " 0 Gy	
E	9 899-6 P		17 899-6 6°		25 None 6°		33 3616 6°	
F	10 " 0 Gy "		18 " 5 Gy		26 " 5 Gy		34 " 6°	
G	11 ~~None~~ 899-6 0°		19 899-6 P		27 None P		35 3616 P	
H	12 " 5 Gy "		20 " 5 Gy		28 " 5 Gy		36 " 5 Gy	

[TNF] By Virus Type (10 exp5 PFU) and RT Dose in U-87 Cells

Virus	Dose	Time	[TNF]	Total [TNF]
899-6	0	0	0.19	62.52
899-6	0	2	2.34	
899-6	0	4	4.99	
899-6	0	6	11.04	
899-6	0	p	10.94	
899-6	5	0	0.94	64.37
899-6	5	2	1.49	
899-6	5	4	4.99	
899-6	5	6	12.29	
899-6	5	p	10.04	
None	0	6	6.64	42.62
None	0	p	1.39	
None	5	6	3.64	24.42
None	5	p	1.29	
R3616	0	6	5.44	42.92
R3616	0	p	5.14	
R3616	5	6	11.09	
R3616	5	p	5.34	

MOLECULAR DEVICES Vmax KINETICS MICROPLATE READER **PLATE #:** 3

ASSAY : ______________________________ DATE: ____/____/____

OPERATOR: ______________________ **O.D. LIMIT:** 3.000 TIME: ____:____AM/PM

WAVELENGTH: 450nm - OPT 2 **READ MODE:** OPTICAL DENSITY **AUTO MIX:** ON

NOTES: ______________________________ **CAL:** ON

OPTICAL DENSITY

	25	26	27	28	29	30	31	32	33	34	35	36
A	0.063	0.176	0.073	0.066	-0.000	0.000	0.000	-0.000	-0.000	-0.000	-0.000	0.000
B	0.081	0.070	0.095	0.132	-0.000	-0.000	-0.000	0.000	-0.000	-0.000	-0.000	-0.000
C	0.067	0.076	0.080	0.077	-0.000	-0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000
D	0.068	0.074	0.090	0.113	-0.000	-0.000	-0.000	0.000	-0.000	-0.000	-0.000	-0.000
E	0.073	0.079	0.095	0.120	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	-0.000
F	0.101	0.077	0.106	0.158	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	-0.000	-0.000
G	0.076	0.068	0.096	0.094	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
[illegible]	0.063	0.070	0.091	0.083	-0.001	-0.001	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000

$(y - .0643)/.005$

$y = .005x + .0643$

$y = .0657e^{.6604x}$

$\frac{\ln y - \ln .0657}{.6604} = \cancel{.6604}x$

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — PLATE #: 1

ASSAY : V87 — DATE: __

OPERATOR: Greg — O.D. LIMIT: [illegible] — TIME: ___:___

WAVELENGTH: 450nm - OPT 2 — READ MODE: OPTICAL DENSITY — AUTO MIX: [illegible]

NOTES: ______ — CAL: [illegible]

Standards

OPTICAL DENSITY

Lisa & Helene's Stuff

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.071	0.086	0.097	0.101	0.092	0.093	0.080	0.073	0.090	0.072	0.127	0.101
B	0.129	0.144	0.070	0.072	0.375	0.366	0.272	0.236	*	*	[illegible]	0.091
C	0.199	0.198	*	*	[illegible]	0.396	0.490	[illegible]	[illegible]	1.202	0.366	[illegible]
D	0.371	0.367	0.343	0.342	0.088	0.086	0.161	0.140	1.578	1.448	0.063	[illegible]
E	0.670	0.727	0.154	0.153	0.237	0.288	*	*	1.205	1.191	0.127	[illegible]
F	1.333	1.385	0.660	0.680	0.647	0.635	0.102	0.110	2.623	2.660	0.198	0.166
G	2.607	2.460	*	*	1.774	1.877	0.129	0.085	0.728	0.700	0.121	0.116
	*	*	0.203	0.193	2.161	2.483	0.066	0.063	0.131	0.039	0.600	0.622

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — PLATE #: [illegible]

ASSAY : ______ — DATE: ___

OPERATOR: ______ — O.D. LIMIT: [illegible] — TIME: ___:___

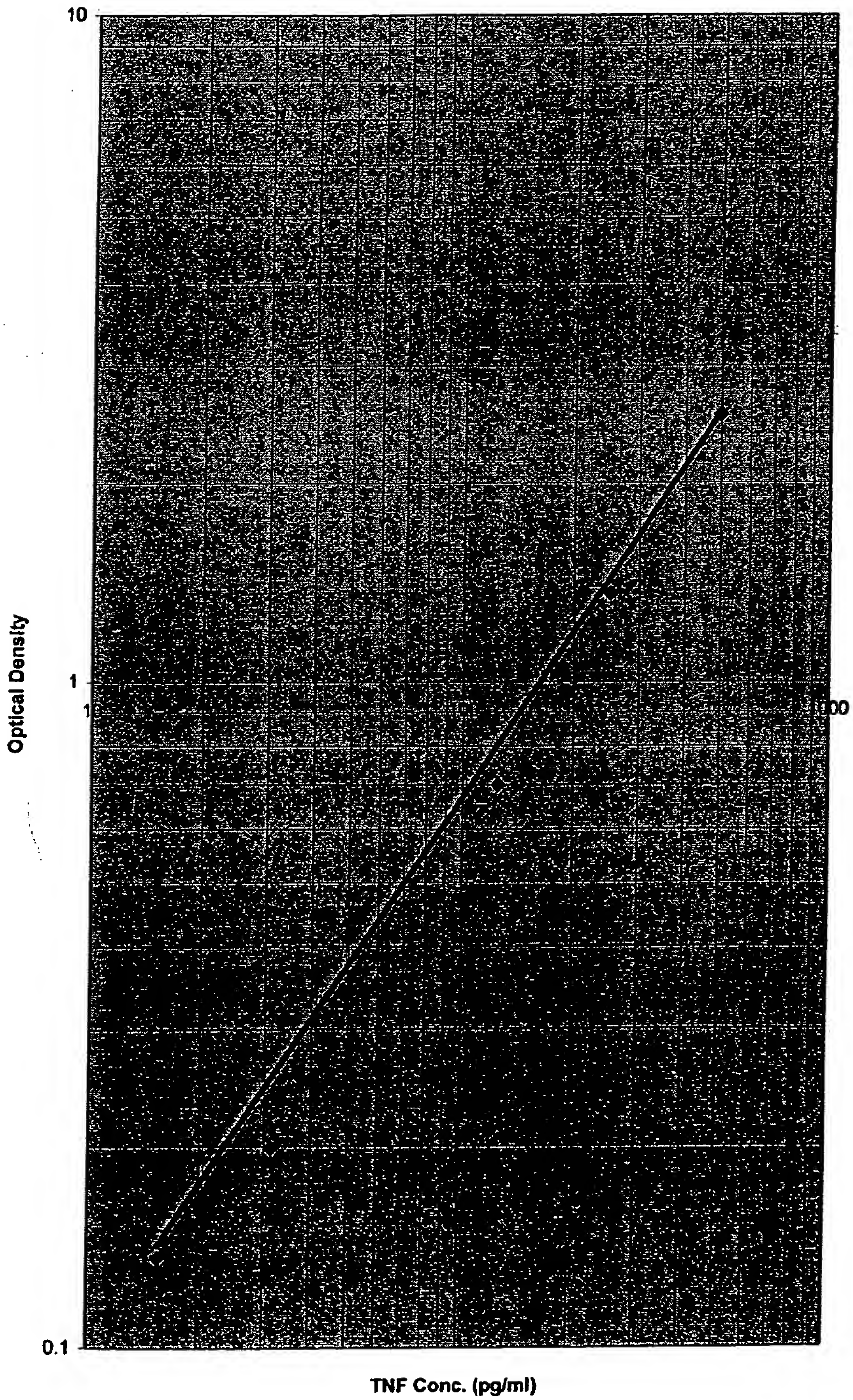
WAVELENGTH: 450nm - OPT 2 — READ MODE: OPTICAL DENSITY — AUTO MIX: [illegible]

NOTES: ______ — CAL: [illegible]

OPTICAL DENSITY

	13	14	15	16	17	18	19	20	21	22	23	24
A	0.075	0.070	0.143	0.160	0.327	0.329	[illegible]	0.192	0.097	0.087	0.077	0.070
B	0.225	0.287	0.145	0.180	0.137	0.151	0.106	0.105	0.094	0.083	0.072	0.064
C	0.810	0.841	0.178	0.609	0.074	0.075	0.219	0.211	0.127	0.117	0.088	0.092
D	0.250	0.253	0.348	0.256	0.128	0.125	0.064	0.063	0.111	0.120	0.096	0.091
E	0.583	0.716	0.079	0.082	0.083	0.097	0.065	0.060	0.109	0.122	0.120	0.125
F	0.126	0.134	0.123	0.183	0.046	0.048	0.064	0.070	0.124	0.121	0.130	0.128
G	0.271	0.242	0.758	0.102	0.051	0.062	0.062	0.073	0.067	0.071	0.109	0.112

TNF ELISA Plot



U 87
R899-6

PURPOSE – Generate cell survival curve for U-87 cells – with different moi's of 899-6 virus

Design – Fixed variables – cell type U87
– intervals = infect x 2°
– virus 899-6

Study variables – moi: 0, 10^{-2}, 10^{-1}, 0.5, 1, 2, 3, 5

U87

METHODS –

① Grow U87 cells to subconfluence on 60 mm plates
(10^6 plated.)

② Subculture & count a plate = 2×10^6
899-6 titer = 1.2×10^9 PFU/ml (from titer)

moi 5 = $1\times10^7 / 1.2\times10^7$ = .83 ml
moi 3 = $6\times10^6 / 1.2\times10^7$ = .5
moi 2 = $4\times10^6 / 1.2\times10^7$ = .33
moi 1 = $2\times10^6 / 1.2\times10^7$ = .17
moi 0.5 = $1\times10^6 / 1.2\times10^6$ = ~~.083~~ .83
moi 0.1 = $2\times10^5 / 1.2\times10^6$ = .17
moi 10^{-2} = $2\times10^4 / 1.2\times10^5$ = .17

③ Infect x 2° in 1 ml 199V

④ Subculture to 100 mm plates c̄ __ ml conditioned media per plate

⑤ Stain & count p̄ ~ 3 wks (18-21 d)

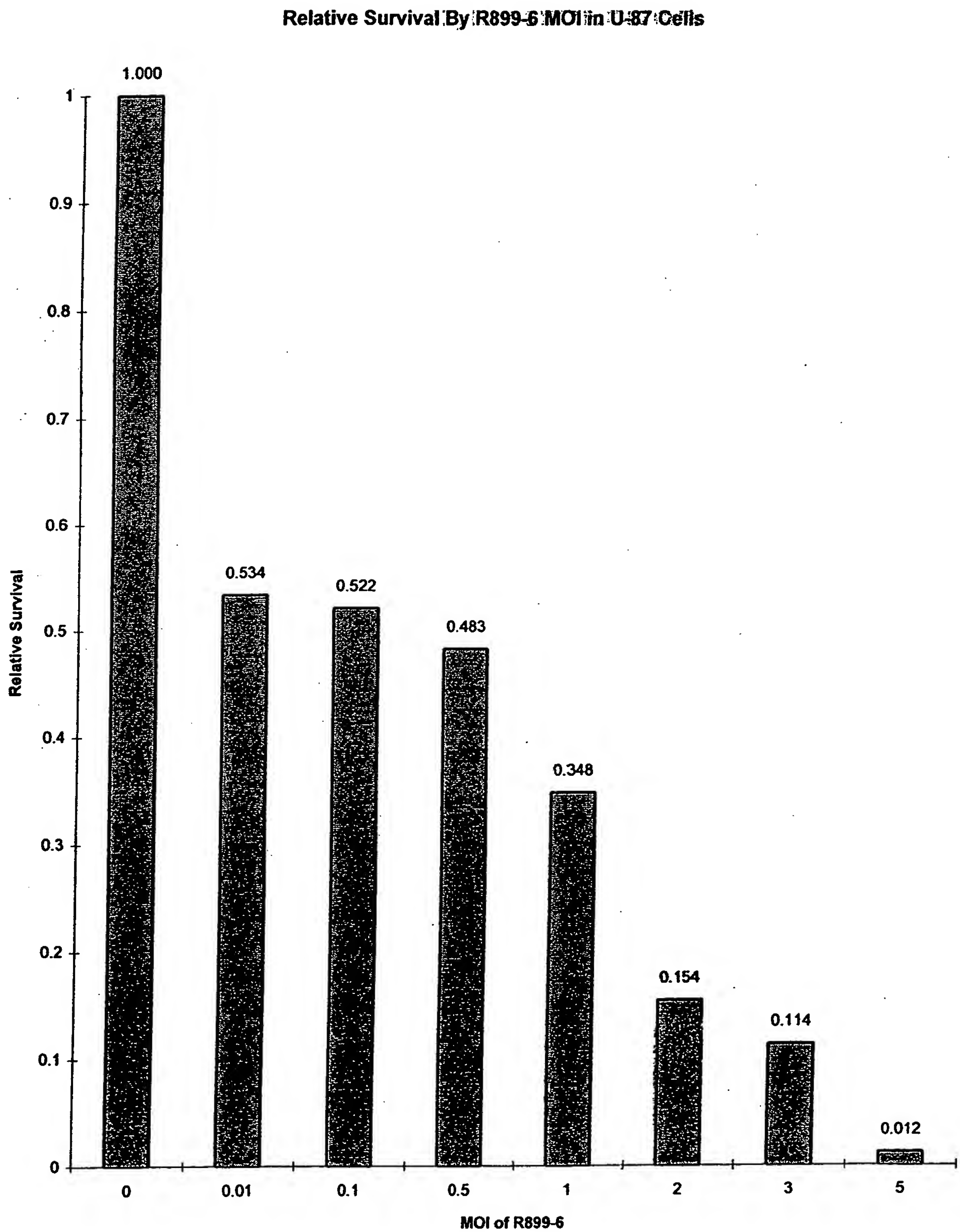
V-87

Results

899-6 moi	# plated	# counted			ave surv.	P.E.	rel surv
0	2000	21	12	19			
	5000	68	64	59			
10^{-2}	2000	5	6				
	5000	53	28	48			
10^{-1}	2000	4	11	6			
	5000	30	50	35			
0.5	5000	34	22	21			
	10^4	51	70	35			
1	10^4	36	37	24			
	2×10^4	90	83	80			
2	10^4	18	13	17			
	2×10^4	33	25	44			
3	2×10^4	18	17	14			
	5×10^4	69	84	89			
5	5×10^4	7	8	5			
	10^5						

Survival of U-87 Cells by MOI of R899-6

MOI	**# plated**	**# counted**			**Ave. Surv.**	**P.E.**	**Rel. Surv.**
0	2000	21	12	19	0.0107	0.0107	1
	5000	68	64	59			
0.01	2000	5	6	6	0.005717	0.0107	0.534268
	5000	53	28	48			
0.1	2000	4	11	6	0.005583	0.0107	0.521807
	5000	30	50	35			
0.5	5000	34	22	21	0.005167	0.0107	0.482866
	10000	51	70	35			
1	10000	36	37	24	0.003725	0.0107	0.348131
	20000	90	83	80			
2	10000	18	13	17	0.00165	0.0107	0.154206
	20000	33	25	44			
3	20000	18	17	14	0.001215	0.0107	0.113551
	50000	69	84	89			
5	50000	7	8	5	0.000133	0.0107	0.012461
MOI	**Rel. Surv.**						
0	1.000						
0.01	0.534						
0.1	0.522						
0.5	0.483						
1	0.348						
2	0.154						
3	0.114						
5	0.012						


Relative Survival By R899-6 MOI in U-87 Cells
1.000
0.534
0.522
0.483
0.348
0.154
0.114
0.012
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Relative Survival
0
0.01
0.1
0.5
1
2
3
5
MOI of R899-6

U-87 Mycoplasma assay

PURPOSE - Rule out mycoplasma infection as reason for poor plating efficiency c̄ U87 cells

METHODS - See attached protocol

CONCLUSION - No mycoplasma infection

* U-87 - TNF

1	2	3	4	5	6	7	8	9

Purpose - Quantify TNF production in U87 cell line
c̄ R3616, 899-6 & Ø virus c̄ 0 or 9 Gy
~~(moi of virus = 0.5)~~ Use 2×10^6 PFU (~moi = 1)

METHODS -
① Plate U87 cells in 60 mm dishes & grow to subconfluen
② 899-6: 2×10^6 PFU / 1.2×10^9 PFU/ml = .17 ml
3616: 2×10^6 PFU / 2.3×10^6 PFU/ml = .87 ml
③ Infect x 2° in total volume 1 cc 199V media
③ Remove inoculum & add 6 ml of 10% FCS media
~~④ Aliquot 1 ml media @ 2, 6~~
④ Irradiate c̄ 9 Gy RT 11am 3pm 9pm 9am 3pm
⑤ Aliquot 1 ml media @ 2°, 6°, 12°, 24°, 30°
then subculture in 3 ml and save 1 ml as pellet

V-87 - TNF

RESULTS —

Virus	RT	time	[TNF]	total [TNF]	Virus	RT	time	[TNF]	total [TNF]
Ø	0	0°			3616	0	0°		
		6°					6°		
		12°					12°		
		24°					24°		
		30°					30°		
		P					P		
Ø	9Gy	0°			3616	9Gy	0°		
		6°					6°		
		12°					12°		
		24°					24°		
		30°					30°		
		P					P		
899-6	0	0°							
		6°							
		12°							
		24°							
		30°							
		P							
899-6	9Gy	0°							
		6°							
		12°							
		24°							
		30°							
		P							

TNF ELISA Assay: U-87 Cells Infected with R899-6, R3616, or No Virus (MOI=0.5) +/- 9 Gy RT

Standards

reading 1	reading 2	[TNF]	ave. reading
0.087	0.087	15.6	0.087
0.119	0.137	31.3	0.128
0.205	0.217	62.5	0.211
0.39	0.407	125	0.399
0.733	0.756	250	0.745
1.354	1.459	500	1.407
1.997	2.284	1000	2.141

				Virus	Dose	Time	Total [TNF]
0.044	0.044	7.8	0.044	None	0	0	62.60
0.045	0.046	8.1	0.046	None	0	10	
0.04	0.046	7.6	0.043	None	0	21	
0.044	0.042	7.6	0.043	None	0	27	
0.043	0.047	8.0	0.045	None	0	P	
0.04	0.048	7.8	0.044	None	9	0	67.73
0.051	0.046	8.8	0.049	None	9	10	
0.052	0.054	9.8	0.053	None	9	21	
0.051	0.048	9.0	0.050	None	9	27	
0.043	0.045	7.8	0.044	None	9	P	
0.047	0.041	7.8	0.044	R3616	0	0	72.24
0.047	0.044	8.1	0.046	R3616	0	10	
0.042	0.055	8.8	0.049	R3616	0	21	
0.054	0.059	10.6	0.057	R3616	0	27	
0.05	0.047	8.8	0.049	R3616	0	P	
0.046	0.042	7.8	0.044	R3616	9	0	67.48
0.046	0.04	7.6	0.043	R3616	9	10	
0.051	0.05	9.2	0.051	R3616	9	21	
0.046	0.048	8.4	0.047	R3616	9	27	
0.048	0.048	8.7	0.048	R3616	9	P	
0.054	0.05	9.6	0.052	R899-6	0	0	3004.23
1.476	1.587	633.2	1.532	R899-6	0	10	
1.836	1.48	698.6	1.658	R899-6	0	21	
1.752	1.973	806.9	1.863	R899-6	0	27	
0.08	0.08	16.3	0.080	R899-6	0	P	
0.047	0.051	8.9	0.049	R899-6	9	0	3323.99
0.939	*0.829*	*320.5*	*0.884*	*R899-6*	*9*	*6*	
1.503	1.569	635.5	1.536	R899-6	9	10	
1.83	1.96	824.4	1.895	R899-6	9	21	
2.076	1.99	899.4	2.033	R899-6	9	27	
0.09	0.089	18.8	0.090	R899-6	9	P	

V-87 ~~TNF~~
R3616

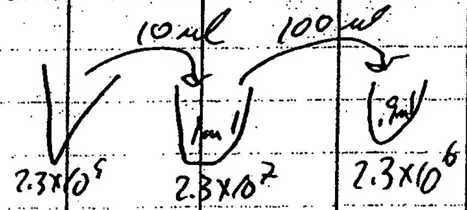
Purpose – Survival of U87 cells c̄ R3616 ~~8T1-6~~ @ MOI of 0 (control) & 0.5 c̄ RT doses: 0, 3, 5, 7, 9 Gy

METHODS –

(1) Plate 10^6 cells in 60 mm dish (12/9) & grow to confluence
(2) Count a dish & infect one dish c̄ MOI 0.5 of ~~8T1-6~~ R3616
(3) Infect x 2° in 1 ml 199V @ 37°
(4) Substitute into 100 mm plates c̄ 50% Cond. Media (+ 5g G). Wait 6°.
(5) Irradiate @ 0, 3, 5, 7, 9 Gy
(6) Incubate x 3 wks
(7) Stain & Count
~~(8) Aliquot media @ 1wk for [TNF]~~
~~(? is there induction c̄ ↑ doses RT~~

Dish count = 2.3×10^6 cells

R3616 titer = 2.3×10^9 PFU/ml



Use 0.5 cc 2.3×10^6 dilution = MOI 0.5

V87

Results -

Virus	RT	# plated	# counted		ave survival	P.E.	rel survival
0	0	2000	13	7			
		5000	40	35			
	3	5000	0	0			
		10^4	12	18			
	5	10^4	8	8			
		2×10^4	28	46			
	7	10^4	5	1			
		2×10^4	6	9			
	9	2×10^4	4	1			
		5×10^4	3	0			
0.5	0	5000	3	2			
		10^4	5	7			
	3	10^4	2	0			
		2×10^4	4	2			
	5	2×10^4	1	0			
		5×10^4	4	8			
	7	2×10^4	1	0			
		5×10^4	5	2			
	9	5×10^4	0	2			
		10^5	0	5			

U-87
R899-6

PURPOSE - Generate cell survival curve for U-87 cells with RT doses 0, 5, 9 Gy & MOI's of 899-6 = 0.5, 0, 0

METHODS ① Plate 10^6 cells per 60 mm dish = U-87

② When subconfluent (2 days) infect c̄ virus in 1cc 199V x 2°.

~~MOI~~ Titer R899-6 = 1.2×10^9 pfu/ml

10 ul → 1 ml → 4.5 ml

1.2×10^9 1.2×10^7 1.2×10^6

MOI = 1 = ~~1.2×10^5~~ $1.6 \times 10^6 / 1.2 \times 10^7 = 0.133$

MOI = 0.5 = $0.8 \times 10^6 / 1.2 \times 10^6 = .667$

~~MOI = 0.1 = $1.6 \times 10^5 / 1.2 \times 10^6$ = .133~~

③ Subculture into 10 mm dishes at appropriate concentration c̄ 50% Conditioned media.

④ Let cells adhere x 6°

⑤ Irradiate

⑥ Incubate x 3 wks, Stain & count.

U-87

Results -

899-6 moi	RT dose	# plated	# counted		ave. survival	P.E.	rel survival
0	0	2000	80	100			
		5000	159	165			
	5	10^4	32	29			
		2×10^4	72	95			
	9	2×10^4	7	6			
		5×10^4	12	8			
0.1	0	2000	40	58			
		5000	81	93			
	5	10^4	14	18			
		2×10^4	46	43			
	9	2×10^4	1	2			
		5×10^4	0	—			
0.5	0	5000	71	73			
		10^4	104	117			
	5	2×10^4	39	50			
		5×10^4	—	—			
	9	5×10^4	0	1			
		10^5	4	3			
1	0	10^4					
		2×10^4					
	5	2×10^4					
		5×10^4					
	9	5×10^4					
		10^5					

PURPOSE – Assess toxicity of R899-6 & R3616 in nude mice

METHODS –

① 8 mice

R899-6: 10 μl of 1.2×10^9 PFU/ml = 1.2×10^7 PFU (2 mice)
10 μl of 1.2×10^8 PFU/ml = 1.2×10^6 PFU (2 mice)

R3616: 10 μl of 2.3×10^9 PFU/ml = 2.3×10^7 PFU (2 mice)
10 μl of 2.3×10^8 PFU/ul = 2.3×10^6 PFU (2 mice)

Cage AA104551 –	1681	Ø	R899-6	1.2×10^7
	1682	L	"	"
	1683	R	"	1.2×10^6
	1684	LR	"	"
Cage AA104552 –	1685	Ø	R3616	2.3×10^7
	1686	L	"	"
	1687	R	"	2.3×10^6
	1688	LR	"	"

② Injected 3:00 pm 1/5/94

RESULTS –

No local or systemic effects noted
Mice examined twice weekly for weeks
Sac'd on

U-87 – plating efficiency

PURPOSE – To improve plating efficiency of U87 cell line by trying different media

METHODS –

Base media	% FCS	CM	# plated	# counted	
MEM/Napyr/aa	10%	+	1000	infected	infected
"	10	+	5000	infected	infected
"	15	+	1000	159	infected
"	15	+	5000	infected	infected
"	20	+	1000	infected	infected
"	20	+	5000	tm	infected
DME – F12	10%	+	1000	infected	infected
"	10	+	5000	Tm	tm
"	15	+	1000	91	infected
"	15	+	5000	tm	tm
"	20	+	1000	131	50
"	20	+	5000	tm	infected

EFFICIENCY LINE® 22-206 AMPAD

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

As per our telephone conversation on please place the following mice on TMP-SMZ (Bactrim) for 1 week:

> Cages AA105283, AA104542 through AA104545, AA104547 through AA10450, and AA107678 through AA107710.

This should include **all** mice from Hallahan's Lab (Radiation Oncology) currently in Cummings Room #1053.

Thank you. If you have any questions you can contact me at beeper #3439 or contact Helena at 2-0294.

Sincerely,

Gregory S. Sibley, M.D.

THE UNIVERSITY OF CHICAGO ANIMAL RESOURCES CENTER ANIMAL PROCUREMENT REQUEST (#315B)

REQ NO 18777

ARC USE ONLY

P.O. # ____

ORDER DATE ____

REF. # ____

CONTACT ____

EST AMT ____

SCHED DEL ____

SPECIAL ROUTING

NON COM VENDOR ____

FLAGGED BY PROTOCOL ____

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ____ SHIPMENTS ON A ____ BASIS

REQUEST BY: Gregory Sibley, MD DATE: ____

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: Malcolm R. Alexander

2-73731-5100

FAS ACCOUNT: ____ P.I.: Hallahan

VENDOR: FCRI PROTOCOL: 58671

REQUESTED DELIVERY DATE: ____ PHONE: ~~2-6819~~ 2-0294

SPECIES: Mouse QUANTITY: 60

STRAIN: Athymic Nude SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE: ____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS: ____

HOUSE AT: ____ CARLSON ____ WYLER X CLSC ____ FMI ____ OTHER

U87 TNF (intervals)

PURPOSE - To determine optimal interval from infex (R899-6) to RT (9 Gy) to optimize TNF production in U87 cells

METHODS -

① Plate 10^6 U87 cells per 60 mm dishes. Incubate ~ 36° for ~ 2×10^6 cells

② Infect plates with 10^6 PFU of R899-6 at
12° prior to RT = 10 pm
8° prior to RT = 2 Am
4° " " " = 6 Am (Actually 6^{45} = 3.25 hrs
2° " " " = 8 Am

Titer R899-6 = 1.2×10^9 PFU/ml
∴ Use 0.83 ml of 1.2×10^6 PFU/ml dilution
+199V media to total vol = 1.5 cc

③ Add 2.5 ml 10% FCS media ⊕ IgG (total vol 4 cc)
"Control" dishes ⇒ Aliquot 1 ml as "supernatant"
~~Try~~ Decant remaining media
Trypsinize cells c̄ 1 ml trypsin + 1 ml Verse
then add 2 ml media (total vol = 4.
Aliquot 1 ml as "pellet"

④ For "treatment" dishes RT c̄ 9 G
Wait 7° then repeat aliquot procedure for supernatant & pellet as above

TNF ELISA **U-87 Cells**

reading 1	reading 2	average	[TNF]	Time	Sample	Average	Total [TNF]	Rel. [TNF]
1.267	1.308	1.2875	325.978	12 hours	control-super	1283.22	1623.47	1239.04
1.248	1.256	1.252	315.632	12 hours	control-super			
0.38	0.353	0.3665	76.5162	12 hours	control-pellet	340.25		
0.474	0.399	0.4365	93.6086	12 hours	control-pellet			
2.331	2.31	2.3205	643.123	12 hours	9 Gy-super	2394.87	2862.506	
2.053	2.027	2.04	554.311	12 hours	9 Gy-super			
0.505	0.534	0.5195	114.425	12 hours	9 Gy-pellet	467.638		
0.537	0.541	0.539	119.394	12 hours	9 Gy-pellet			
0.417	0.422	0.4195	89.416	8 hours	control-super	360.864	642.5502	2071.4
0.421	0.431	0.426	91.016	8 hours	control-super			
0.311	0.34	0.3255	66.73	8 hours	control-pellet	281.686		
0.343	0.37	0.3565	74.113	8 hours	control-pellet			
1.455	1.503	1.479	382.519	8 hours	9 Gy-super	1953.96	2713.952	
2.127	2.208	2.1675	594.462	8 hours	9 Gy-super			
0.587	0.607	0.597	134.332	8 hours	9 Gy-pellet	759.989		
0.957	1.058	1.0075	245.662	8 hours	9 Gy-pellet			
0.065	0.058	0.0615	9.76247	3.25 hours	control-super	40.8921	130.1639	1371.14
0.065	0.068	0.0665	10.6836	3.25 hours	control-super			
0.085	0.087	0.086	14.3726	3.25 hours	control-pellet	89.2718		
0.158	0.17	0.164	30.2632	3.25 hours	control-pellet			
0.933	0.991	0.962	232.909	3.25 hours	9 Gy-super	906.04	1501.304	
0.901	0.931	0.916	220.111	3.25 hours	9 Gy-super			
0.709	0.714	0.7115	164.467	3.25 hours	9 Gy-pellet	595.264		
0.599	0.586	0.5925	133.165	3.25 hours	9 Gy-pellet			
0.059	0.068	0.0635	10.1296	2 hours	control-super	41.0714	131.0586	2360.16
0.065	0.065	0.065	10.4061	2 hours	control-super			
0.067	0.112	0.0895	15.0494	2 hours	control-pellet	89.9873		
0.179	0.146	0.1625	29.9442	2 hours	control-pellet			
1.414	1.524	1.469	379.538	2 hours	9 Gy-super	1679.41	2491.216	
1.687	1.785	1.736	460.167	2 hours	9 Gy-super			
0.859	0.824	0.8415	199.593	2 hours	9 Gy-pellet	811.806		
0.866	0.866	0.866	206.311	2 hours	9 Gy-pellet			

U-87 TNF (intervals)

PURPOSE - To determine optimal interval from infxn (R899-6) to RT (9Gy) to optimize ~~TNF production~~ subsequent TNF induction experiments

METHODS -

① Plate 10^6 U87 cells per 60 mm dish. Incubate ~~48°~~ ~36° for ~2×10^6 cells

② Infect plates $\bar{c}$ 10^6 PFU R899-6
= 0.83 ml of 1.2×10^6 PFU/ml dilution
+ 199V media to ≈ 1.5 ml

Infect @ 7^{30} AM

③ Aliquot & trypsinize "control dishes" @
4° $\bar{p}$ infection 11^{30}
6° " " 1^{30}
8° " " 3^{30}

④ Irradiate @ same time points $\bar{c}$ 9 Gy

⑤ Aliquot & trypsinize "treatment dishes" @
7° $\bar{p}$ RT = 6^{30} PM, 8^{30} PM, 10^{30} PM

In Vivo - TNF induction - intervals
U87 hind limbs nude mice

Purpose - To determine optimal interval from infection → RT in U87 tumors in hind limbs of nude mice for TNF production

Methods -

① Transplant $1 mm^3$ U87 tumors into hind limbs of nude mice. Grow to ~ 6 mm (range 4.5-8 mm) greatest dimension.

② Infect mice in groups of 4 @ 48°, 24°, 10° & 5° prior to RT.

- $-48° = 4^{pm}$ 1/24
- $-24° = 4^{pm}$ 1/25
- $-10° = 7^{AM}$ 1/26
- $-5° = 12^{N}$ 1/26
- $0° = 5^{pm}$ 1/26 = Irradiate c̄ 20 Gy single fraction

③ Sacrifice mice @ 4^{pm}

Greg's Mouse Log

	Mouse #	Cage #	DOB	Tumor Date	Infect Date	RT Date	Date Sacrif	Tumor size	
	1703	~~AA104542~~			R			7.5	8×5×3.5
	1711	~~AA104542~~			Ø OK			6	5.5×5×2.5
24°	1712	~~AA104542~~			L			8	10×8.5×6
	1714	~~AA104542~~			LR			6	6×6×3.5
	1701	~~AA104543~~			Ø			6.5	7.5×6×4
8°	1715	~~AA104543~~			Ø → L			4.5	5.5×5×3
	1718	~~AA104543~~			LR			5.5	4×5.5×2.5
	1721	~~AA104543~~			Ø → R			7	6×7.5×3
	1716	AA104544						3.5	
	1722	AA104544						3.5	
	1723	AA104544						3	
	1724	AA104544						2	
	~~1689~~	~~AA104546~~							
	~~1690~~	~~AA104546~~							
	~~1691~~	~~AA104546~~							
	~~1692~~	~~AA104546~~							
	1693	AA104547			Ø			10	
	~~1694~~	~~AA104547~~						20	
	1695	AA104547			SaC			0	
	1696	~~AA104548~~			LR			6	6.5×5.5×3.5
	1698	~~AA104548~~			L			4	9×9.5×8
	1699	~~AA104548~~			R			5	6×5×3
48°	1700	~~AA104548~~			LR → LRR			6	4.5×2.5×2
	1697	AA104549						3.5	
	1702	AA104549						2	
	1713	AA104549						4.5	
	1717	AA104549						2.5	
	1704	AA104550						3.5	
	~~1705~~	~~AA104550~~							
	1706	AA104550			SaC			0	
	1719	AA104550						3.5	
	1720	AA104550						3.5	
	1681	AA104551							
	1682	AA104551							
	1683	AA104551							
	1684	AA104551							
	1685	AA104552							
	1686	AA104552							
	1687	AA104552							
	1688	AA104552							
	1707	AA105283						0	
	1708	AA105283						3	
	1709	AA105283						2	
	1710	AA105283						3	

$$\frac{4}{3}\pi r^3$$

$$\frac{4}{3}\pi\left(\frac{d}{2}\right)^3$$

$$\frac{\cancel{4}}{\cancel{6}}\,\cancel{3}\pi\,\frac{d^3}{\cancel{8}\,2}$$

$$\frac{d^3}{2}$$

5170 [illegible]

1867	23	20.5	12	25.1
1868	23	24	20.5	22.3
1869	21	23	15	24.2
1870	20	18	13	26.9

4550 Control

1719	20	14.5	10	21.8
1722	27	23	19	25.9
1717	17	15.5	12	23.7

5283 391-6

1710	10.5	1.5	3	20.7
1713	0	0	0	22.0
1720	11.5	1.5	.5	26.0

4549 3016

1617	14.5	12	7.5	26.3
1723	0	0	0	27.1
[illegible]09	5.5	6	1.5	25.8
1708	3	3.5	2	21.0

8963 3016

1875	17.5	11.5	7	22.3
1876	11	10.5	10	24.9
1877	[illegible]	[illegible]	11	25.5
1878	15	15	10	22.0

8972 2T

1859	15	10	0	22.8
1860	13	11.5	6	19.0
1861	10	12	5	21.1
1862	9	9	3.5	23.3

8964 2T

[illegible]	7	7	5.5	19.0
1892	12.5	12.5	7	23.1
1893	10.5	15	7	22.4
1894	9	5.5	5	21.4

8969 391-6+2T

1872	7	7	5	23.7
1872	[illegible]	6	4	22.2
1873	9	7.5	5.5	22.2
1874	9.5	15	7	26.0

8967 391-6+2T

1887	2	1	0	24.5
1890	3.5	3	1	20.2
1891	5	6.5	2	23.9
1892	5.5	3.5	3.5	2[illegible] 7

8971 3616+2T

1863	9.5	8	3.5	19.7
1864	11	7	3	23.0
1865	7	5.5	3.5	24.4
1866	7	15.5	14	25.5

8966 3616+2T

1883	4.5	2.5	2.5	20.7
1884	6.5	10	5	21.0
1885	12	8.5	6	19.6
1886	4	1	2	[illegible]

Day 7					Day 10														
9.5	9.5	5.5	248.19		11	10	6	315	24.5	10	12	6.5	373.75					0	
12	11	8	504		13	12	7	546	18.6	9	8	6.5	234					0	
11	9	7	330.75		12	12	7	504	19.2	12	13	8	575					0	
12	9	7	378		11	8.5	4.5	210.38	21.4	11	9	4	198					0	
	Mean:		365.23			Mean:		393.84			Mean:		345.19			Mean:		0	
	Std Error:		37.809			Std Error:		79.143			Std Error:		60.435			Std Error:		0	
	Day:		7			Day:		10			Day:		14			Day:		17	
Day 7					Day 10														
10	12	6	360	20.5	12	9	6	310.5					0					0	
9	13	5	292.5	21.2	13	8	4.5	225					0					0	
10	7.5	4.5	168.75	22	6.5	8	5	130					0					0	
10	8	5.5	220	22.9	8.5	8	5.5	187					0					0	
6.5	5.5	3.5	62.563	20.2	6	5.5	4	66					0					0	
12	11	7.5	495	21.4	13	11	7	459.38					0					0	
12	9	6	310.5	22.6	12	9.5	5	273.13					0					0	
6	7	4	84	21.5	5.5	5	4	55					0					0	
	Mean:		249.16			Mean:		213.25			Mean:		0			Mean:		0	
	Std Error:		51.37			Std Error:		47.828			Std Error:		0			Std Error:		0	
	Day:		7			Day:		10			Day:		0			Day:		0	
Day 7																			
9	7.5	5.5	185.63	21.3	8	7.5	5.5	165					0					0	
9	7	5	157.5	19.7	8	6.5	5	130					0					0	
9.5	7.5	5.5	195.94	22	8.5	6.5	5	138.13					0					0	
10	9	6	270	23.4	9	9	6	243					0					0	
5	5.5	4	55	24.3	5	4.5	3.5	39.375					0					0	
6.5	5	3	48.75	21.3	5.5	4.5	3	37.125					0					0	
8	7.5	4	120	22.4	7	7	3	73.5					0					0	
7.5	6	3	67.5	20.7	7	5	3	52.5					0					0	
	Mean:		202.27			Mean:		109.83			Mean:		0			Mean:		0	
	Std Error:		23.995			Std Error:		25.649			Std Error:		0			Std Error:		0	
	Day:		7			Day:		10			Day:		0			Day:		0	

8968 1023616

				wt			
1875	13.5	12	7	24.6	15.3	14.5	7.5
1876	12	10.5	8	21.3	13.5	14	[illegible]
1877	10	11	7	23.7	10.5	8.5	7.5
1878	10	8	6	24.3	[illegible]	9	[illegible]

8965 899-6 all mice drowned [illegible]

				wt
1887	9	10.5	6	23.4
1888	10	7	5.5	24.0
1889	4	3.5	1	23.4
1890	6	6	4.5	22.4

8967 899-6 + RT

				wt			
1879	4	5	3	24.6	0	0	0
1880	3	3	2	19.5	0	0	0
1881	7	[illegible]	4	22.7	7	6	4
1882	7	6	4	20.0	[illegible]	4.5	3

[illegible]9 899-6 + RT

				wt			
1871	8	8	5	20.7	7	6	4.5
1872	8	6.5	4.5	20.6	8	[illegible]	4
1873	8.5	9	5.5	20.1	[illegible]	7.5	6
1874	10	8.5	[illegible]	23.6	8	7	4

8966 3616 + RT

				wt			
1883	5	4	3.5	20.6	4	3.5	3.5
1884	10	11	7.5	21.1	11	[illegible]	6
1885	11	10	7.5	23.2	10.5	9	6.5
1886	5	6	3.5	19.4	4	3.5	2.5

8964 RT

				wt.			
1891	7	7.5	6	20.1	7	7	[illegible]
1892	9.5	9	7	24.0	9	7.5	6.5
1893	9	10	7.5	22.5	11	9.5	7
1894	9	9	6	21.8	[illegible]	[illegible]	[illegible]

Greg's Mouse Log

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif	Day 0				weight	Day 3				weight
1704	DD104550	Control		None	None		9.5	8	6	228		12	14	9	698.63	24.8
1717	AA104550	Control		None	None		6	6.5	5.5	107.3		9.5	8	7	266	25.2
1719	AA104550	Control		None	None		7	7	6	147		8	8.5	5	170	21.4
1722	AA104550	Control		None	None		7	6.5	6.5	147.9		9.5	8	4	152	24.5
1867	AA108970	Control		None	None		8	6	5	120	23.7	10	8	6	240	
1868	AA108970	Control		None	None		8.5	7	6.5	193.4	21.2	10	9.5	7.5	356.25	
1869	AA108970	Control		None	None		8.5	7	4.5	133.9	22.7	10	9	7	315	
1870	AA108970	Control		None	None		9	7	6	189	22.7	11	8.5	8	357	
		Control						Mean:		158.3					319.36	
								Std Error:		14.6				r:	60.666	
								Day:		0			Day:		3	
							Day 0					Day 3				
1720	AA105283	10^7 899-6			None		9	9	5.5	222.8	25	12	12	6.5	468	
1710	AA105283	10^7 899-6			None		9	8.5	5.5	210.4	21.7	10	10	9	450	
1707	AA105283	10^7 899-6			None		11	11	5	275.6	20	11	13	9	614.25	
1713	AA105283	10^7 899-6			None		9	9	5	202.5	23	9	10	6.5	292.5	
1887	AA108965	10^7 899-6			None		8.5	6	5	127.5	22.5	9.5	9	5	213.75	22.6
1888	AA108965	10^7 899-6			None		8.5	5.5	4.5	105.2	23.4	9	8	4.5	162	23.2
1889	AA108965	10^7 899-6			None		5.5	8	5	110	22.8	7	5.5	2.5	48.125	23.1
1890	AA108965	10^7 899-6			None		7.5	6.5	5	121.9	23.3	8.8	8	5	176	22.9
		10^7 899-6						Mean:		172			Mean:		303.08	
								Std Error:		22.57			Std Error:		67.448	
								Day:		0			Day:		3	
							Day 0					Day 3				
1697	AA104549	10^7 3616			None		9.5	7.5	7	249.4		11	11	7	423.5	
1708	AA104549	10^7 3616			None		7.5	7	5	131.3		9.5	9	6	256.5	
1709	AA104549	10^7 3616			None		7	8.5	4.5	133.9		9.5	10	6.5	308.75	
1723	AA104549	10^7 3616			None		7	5.5	5	96.25		8.5	8	5.5	187	
1875	AA108968	10^7 3616			None		11	10	7.5	412.5	20.1	13	12	8	598	
1876	AA108968	10^7 3616			None		7.5	8	6	180	24.2	10	9	7.5	337.5	
1877	AA108968	10^7 3616			None		9	7.5	6.5	219.4	24.2	10	9.5	8	380	
1878	AA108968	10^7 3616			None		6.5	8.5	4.5	124.3	24.8	8.5	8	5.5	187	
		10^7 3616						Mean:		193.4			Mean:		334.78	
								Std Error:		36.26			Std Error:		48.124	
								Day:		0			Day:		3	

8971 3613 +RT

				wt.			
1863	11	9	6.5	21.1	10.5	9.5	4.5
1864	11	7	5	21.4	10.5	8	9.5
1865	8	7.5	7.5	22.1	7.5	6.5	5
1866	7	8.5	5	22.7	7	6.5	5

8972 RT

				wt.			
1859	11	11.5	7	23.3	10	10.5	5
1860	11.5	12	8.5	18.6	11.5	12	7.5
1861	12	11	7.5	20.1	12	9.5	6.5
1862	10	8.5	5	23.1	10	8	5

4549 3616

				wt.			
1697	12	9	5.5	25.6	12.5	11	6
1709	8	6	5	27.2	7	7	4
1723	6	6	3.5	25.6	5	5	2.5
1708	5	4	3.5	23.0	3	4	2.5

5083 819-6

1707 died 2/24

				wt.			
1720	11.5	1.5	7.5	26.9	14	11	8
1713	0	0	0	21.7	0	0	0
1710	13.5	17	5.5	21.3	15	12.5	5

8970 control

				wt.			
1867	18	19	10	23.9	20	16	10.5
1868	21	17.5	15.5	21.6	20.5	9.5	18.5
1869	18	16	11	25.5	21	12.5	12
1870	17	15	11	24.6	17.5	6.5	12

4550 control

				wt.			
1719	11.5	16	9	22.0	~~18.5~~ 17.5	13	10
1722	21	20	10.5	25.4	23	22.5	16.5
1717	~~13~~ 15	~~8~~ 15	~~7~~ 18	24.7	17.5	16.5	13.5

				Day 0					Day 3			
1859	AA108972	RT Alone	None	8	9.5	6	228		10	9	6	270
1860	AA108972	RT Alone	None	9.5	11	7	349.1		13	12	7	523.25
1861	AA108972	RT Alone	None	9	8.5	7	267.8		9.5	10	6	285
1862	AA108972	RT Alone	None	10	7	4.5	157.5		12	8.5	7	357
1891	AA108964	RT Alone	None	6.5	6	5	97.5	20.7	6.5	7	5.5	125.13
1892	AA108964	RT Alone	None	7	10	5.5	192.5	23.5	8	9	5	180
1893	AA108964	RT Alone	None	9	6	5	135	23.1	9.5	7.5	7	249.38
1894	AA108964	RT Alone	None	7	7	4.5	110.3	22.4	8	8.5	6	204
		RT Alone			Mean:		192.2			Mean:		274.22
					Std Error:		30.41			Std Error:		43.437
					Day:		0			Day:		3
				Day 0					Day 3			
1863	AA108971	10^7 3616+F		10	7	6.5	227.5	21.3	9	8	6.5	234
1864	AA108971	10^7 3616+F		12	7	5.5	231	21.4	13	7.5	6	281.25
1865	AA108971	10^7 3616+F		8.5	7	5	148.8	22.6	10	9	6.5	292.5
1866	AA108971	10^7 3616+F		9.5	6.5	5.5	169.8	23	11	7.5	5.5	216.56
1883	AA108966	10^7 3616+F		7	6	5.5	115.5	19.6	7.5	7	5	131.25
1884	AA108966	10^7 3616+F		9.5	8	6	228	22	12	10	8	460
1885	AA108966	10^7 3616+F		11	7.5	6.5	255.9	23.8	12	9	7	378
1886	AA108966	10^7 3616+F		6.5	6	4.5	87.75	20.2	7.5	7.5	5	140.63
		10^7 3616+R			Mean:		183			Mean:		266.77
					Std Error:		21.8			Std Error:		39.743
					Day:		0			Day:		3
				Day 0								
1871	AA108969	10^7 899-6+		8	6	5	120	22.2	9	8	5.5	198
1872	AA108969	10^7 899-6+		8.5	7	6	178.5	20.2	9.5	7.5	6.5	231.56
1873	AA108969	10^7 899-6+		8.5	6	6.5	165.8	21.4	9.5	8	7	266
1874	AA108969	10^7 899-6+		11	9	6	283.5	24.5	11	11	8	462
1879	AA108967	10^7 899-6+		6.5	7	5.5	125.1	23.8	8	9	6	216
1880	AA108967	10^7 899-6+		7	6.5	5	113.8	22.2	8	7	6	168
1881	AA108967	10^7 899-6+		7	6	4.5	94.5	23.3	8	7	5	140
1882	AA108967	10^7 899-6+		7.5	6.5	5	121.9	20.4	9.5	8	5	190
		10^7 899-6+R			Mean:		150.4			Mean:		233.95
					Std Error:		21.37			Std Error:		35.295
					Day:		0			Day:		3

~~58~~
56

ELISA

	Standards								
	1	2	3	4	5	6	7	8	9
	0	"	4° 0-S	"	6° 0-S	"	8° 0-S	"	VTM
	15	"	4° 0-S	"	6° 0-S	"	8° 0-S	"	"
	31	"	4° 0-P	"	6° 0-P	"	8° 0-P	"	MED
	63	"	4° 0-P	"	6° 0-P	"	8° 0-P	"	"
	125	"	4° 7-S	"	6° 7-S	"	8° 7-S	"	199V
	250	"	4° 7-S	"	6° 7-S	"	8° 7-S	"	"
	500	"	4° 7-P	"	6° 7-P	"	8° 7-P	"	
	1000	"	4° 7-P	"	6° 7-P	"	8° 7-P	"	

	10	11
8	0	"
7	15	"
6	31	"
5	63	"
4	125	"
3	250	"
2	500	"
1	1000	"

File | Edit Data | Linear Regression | Set fx= | X

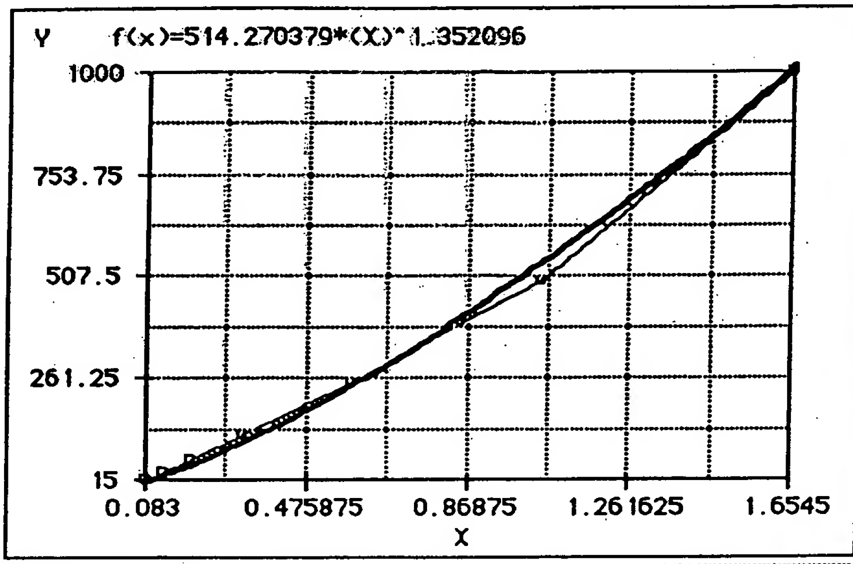
DATA:

X	Y
0.083	15.625
0.128	31.25
0.1925	62.5
0.318	125
0.587	250
1.0455	500
1.6545	1000

• EXPONENTIAL FIT •

Y= 514.270379*(X)^1.352096

Coef. of Regression= 0.995716 For 7 Data Pairs



INITIALS GSS SUBJECT • Untilted

DATE

CPU Time: 12 Secs.

TNF ELISA U-87 Cells

reading 1	reading 2	average	[TNF]/ml	Time	Sample	[TNF]	Total [TNF]	Rel. [TNF]
0.053	0.063	0.058	10.9483	4 hours	control-super	41.0337	131.644	1497.08
0.053	0.052	0.0525	9.56856	4 hours	control-super			
0.096	0.114	0.105	24.4253	4 hours	control-pellet	90.6103		
0.089	0.098	0.0935	20.8799	4 hours	control-pellet			
0.717	0.721	0.719	329.222	4 hours	9 Gy-super	1275.75	1628.722	
0.646	0.725	0.6855	308.655	4 hours	9 Gy-super			
0.281	0.271	0.276	90.2195	4 hours	9 Gy-pellet	352.969		
0.281	0.253	0.267	86.265	4 hours	9 Gy-pellet			
0.111	0.13	0.1205	29.4229	6 hours	control-super	103.653	305.0815	1957.19
0.09	0.107	0.0985	22.4036	6 hours	control-super			
0.179	0.195	0.187	53.2992	6 hours	control-pellet	201.429		
0.177	0.166	0.1715	47.4151	6 hours	control-pellet			
0.875	0.872	0.8735	428.33	6 hours	9 Gy-super	1776.22	2262.27	
0.889	0.952	0.9205	459.781	6 hours	9 Gy-super			
0.344	0.344	0.344	121.512	6 hours	9 Gy-pellet	486.047		
0.36	0.328	0.344	121.512	6 hours	9 Gy-pellet			
0.305	0.332	0.3185	109.495	8 hours	control-super	397.223	660.3809	1933.52
0.27	0.277	0.2735	89.1164	8 hours	control-super			
0.22	0.21	0.215	64.3647	8 hours	control-pellet	263.158		
0.222	0.222	0.222	67.2141	8 hours	control-pellet			
1.084	1.073	1.0785	569.592	8 hours	9 Gy-super	2237.86	2593.9	
1.068	1.032	1.05	549.337	8 hours	9 Gy-super			
0.28	0.259	0.2695	87.3588	8 hours	9 Gy-pellet	356.041		
0.308	0.246	0.277	90.6617	8 hours	9 Gy-pellet			

TNF ELISA

Standards

1	2	3	4	5
0	"	1693 (48°)	"	1701 (8°)
15	"	1696	"	"
31	"	1699	"	1715
63	"	1700	"	"
125	"	1703 (24°)	"	1718
250	"	1711	"	"
500	"	1712	"	1721
1000	"	1714	"	"

WAVELENGTH: READ MODE AUTO MIX

CAL

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
B	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
D	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

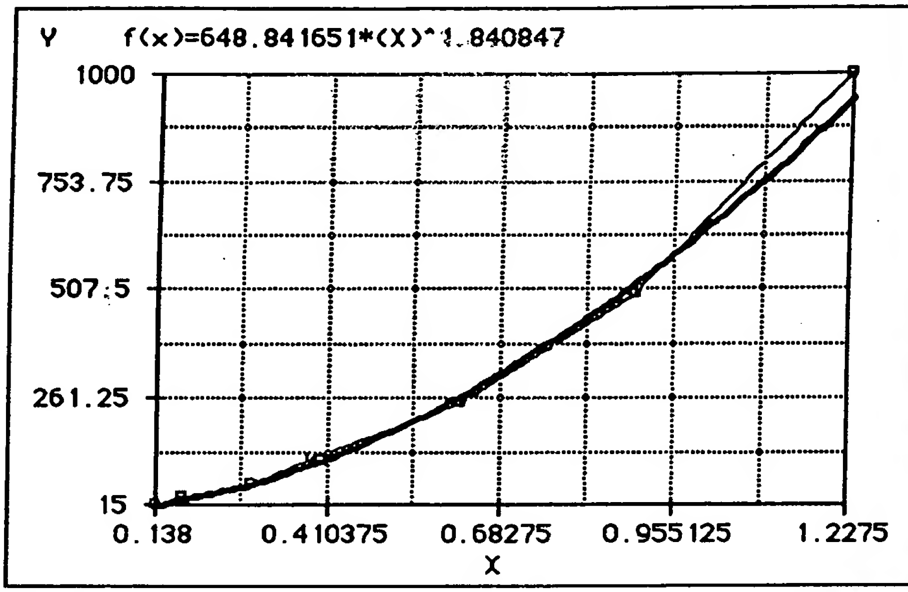
$$y = 648.842 \times x^{1.84}$$

File | Edit Data | Linear Regression | Set fx= | X

DATA:

X	Y
0.138	15.63
0.182	31.25
0.289	62.5
0.3845	125
0.623	250
0.8935	500
1.2275	1000

• EXPONENTIAL FIT •
Y= 648.841651*(X)^1.840847
Coef. of Regression= 0.996618 For 7 Data Pairs



INITIALS G.S.S SUBJECT • Untilted
DATE ? CPU Time: 9 Secs.

TNF ELISA In vivo

reading 1	reading 2	average	[TNF]/ml	Time	Mouse #
0.339	0.207	0.273	59.52191	48 hours	1693
0.117	0.114	0.1155	12.22611	48 hours	1696
1.271	0.47	0.8705	502.7038	48 hours	1699
2.667	1.356	2.0115	2347.548	48 hours	1700
1.744	0.936	1.34	1111.758	24 hours	1703
0.758	0.319	0.5385	207.7396	24 hours	1711
0.328	0.218	0.273	59.52191	24 hours	1712
0.974	0.379	0.6765	316.1043	24 hours	1714
0.85	0.462	0.656	298.7038	8 hours	1701
0.148	0.106	0.127	14.55916	8 hours	1715
1.197	0.631	0.914	549.8935	8 hours	1718
0.572	0.47	0.521	195.4875	8 hours	1721

TNF Interval (Infection with R899-6 to RT with 20 Gy)						
Mouse #	Tumor Size	Mean Size	Interval	[TNF]/ml	Mean [TNF]	[Prot]
1693	342	115.20	48 hours	59.52	730.50	
1696	62.5625		48 hours	12.23		
1699	45		48 hours	502.70		
1700	11.25		48 hours	2347.55		
1703	70	105.59	24 hours	1111.76	423.78	
1711	34.375		24 hours	207.74		
1712	255		24 hours	59.52		
1714	63		24 hours	316.10		
1701	90	56.56	8 hours	298.70	264.66	
1715	27.5		8 hours	14.56		
1718	41.25		8 hours	549.89		
1721	67.5		8 hours	195.49		

Infect $\xrightarrow{\text{I}}$ RT 20Gy $\xrightarrow{24°}$ SEC 20 Gy

I $\xrightarrow{24°}$ RT 20Gy $\xrightarrow{24°}$ RT 25Gy $\xrightarrow{\text{follow}}$

What we have:

① 4 mice injected c̄ 10^6 U87 cells, now c̄ small tumors
② 27 mice implanted c̄ $1 mm^3$ U87 implants
③ 4 mice s̄ tumors injected c̄ R3616
④ 4 " " " " c̄ R899-6

I. Compare 3616 & 899-6 in mice c̄ 1° U87 implants c̄
– Design: alternately inject tumors c̄ 4×10^7 PFU of R3616 or R899-6 when tumors reach 6 mm in greatest dimension.
Measure tumors 2x/wk
Sac mice (i.e. 1 from each group on days 7, 14, 21, 28) for pathology?

II. Use 2° passaged U87 implants (Need 48 mice – order 60)
– Control + saline
– RT (45 Gy single dose) + saline
– R3616 (4×10^7 PFU single dose)
– R899-6 (4×10^7 PFU single dose)
– RT (45 Gy) + R3616
– RT (45 Gy) + R899-6

2×10^9/ml
.025
4×10^7

– Design: Take mice in groups of 4 when they reach 6mm in greatest dimension and randomly assign to a group. Use 8 mice per group.
– Deliver RT 24° (?) p̄ viral inoculation
– Measure tumors 2x/wk

III. In Vivo TNF assay c̄ 2° passaged U87 tumors

- R3616
- R899-6
- R3616 + RT
- R899-6 + RT
- Design = inject tumors when reach 6 mm
  Sac mice & assay tumors for TNF at specific interva
  following inoculation: 12°, 24°, 36°, 48°, 60°
  ((correct for [protein])

IV. Use 4 mice c̄ small tumors inject c̄ cells ①

- Sac & assay tumor for TNF to serve as control for III.

TNF U87

PURPOSE - To optimize intervals in order to test for RT inducibility

METHODS -

1. U87 cells were grown to subconfluence in 60 mm plates
2. Cell were infected with 10^6 PFU of R899-6 in 1.5 ml 199V media for 2°, then 2.5 ml of U87 media + IgG was added (total vol 4 ml)
3. Cells were incubated and irradiated with 8 Gy at the following intervals. The "supernatant" and "pellet" samples were aliquotted as in previous experiments.

Infect @ 6 AM ⇒

RT time	Assay time
None	6, 8, 10, 12, 14°
6°	8, 10, 12, 14°
8°	10, 12, 14°
10°	12, 14°
12°	14°

4. Prepare samples by freeze thaw x 3, then centrifuge
5. Measure human TNFα by ELISA

1	2	3	4	5	6	7	8	9	10
0	"	T_6 s	"	R_8T_{10} s	"	R_8T_{12} s	"	$R_{10}T_{14}$ s	"
15	"	T_6 p	"	R_8T_{10} p	"	R_8T_{12} p	"	$R_{10}T_{14}$ p	"
31	"	T_8 s	"	R_6T_{10} s	"	R_6T_{12} s	"	R_8T_{14} s	"
63	"	T_8 p	"	R_6T_{10} p	"	R_6T_{12} p	"	R_8T_{14} p	"
125	"	R_6T_8 s	"	T_{12} s	"	T_{14} s	"	R_6T_{14} s	"
250 *	"	R_6T_8 p	"	T_{12} p	"	T_{14} p	"	R_6T_{14} p	"
500	"	T_{10} s	"	$R_{10}T_{12}$ s	"	$R_{12}T_{14}$ s	" *		
1000	"	T_{10} p	"	$R_{10}T_{12}$ p	"	$R_{12}T_{14}$ p	"		

PLATE #

TNF

Sibley/Hyland

WAVELENGTH AUTO MIX

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												

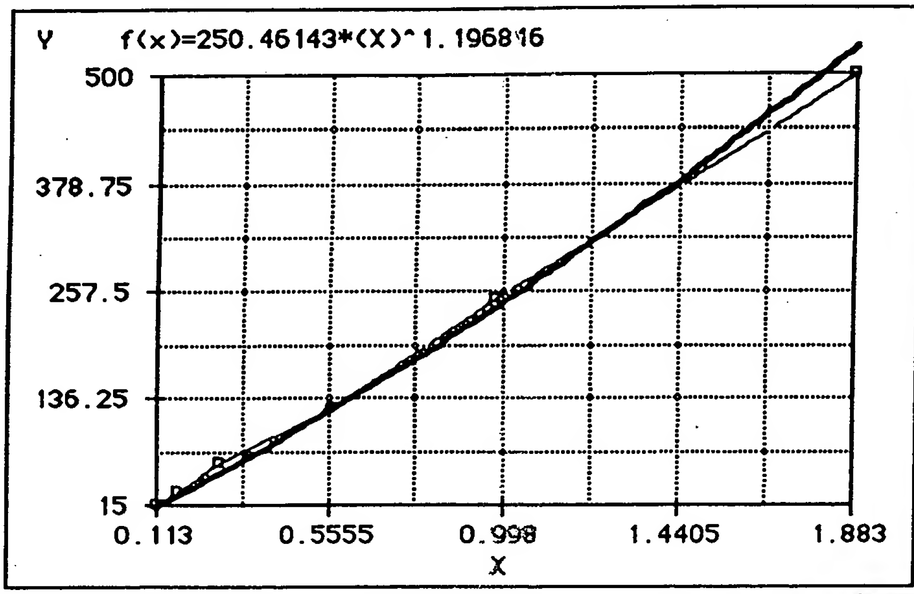
$$y = 250.46 * X^{1.1968}$$

File | Edit Data | Linear Regression | Set fx= | X

DATA:

X	Y
0.113	15.625
0.1645	31.25
0.2785	62.5
0.5645	125
0.981	250
1.883	500

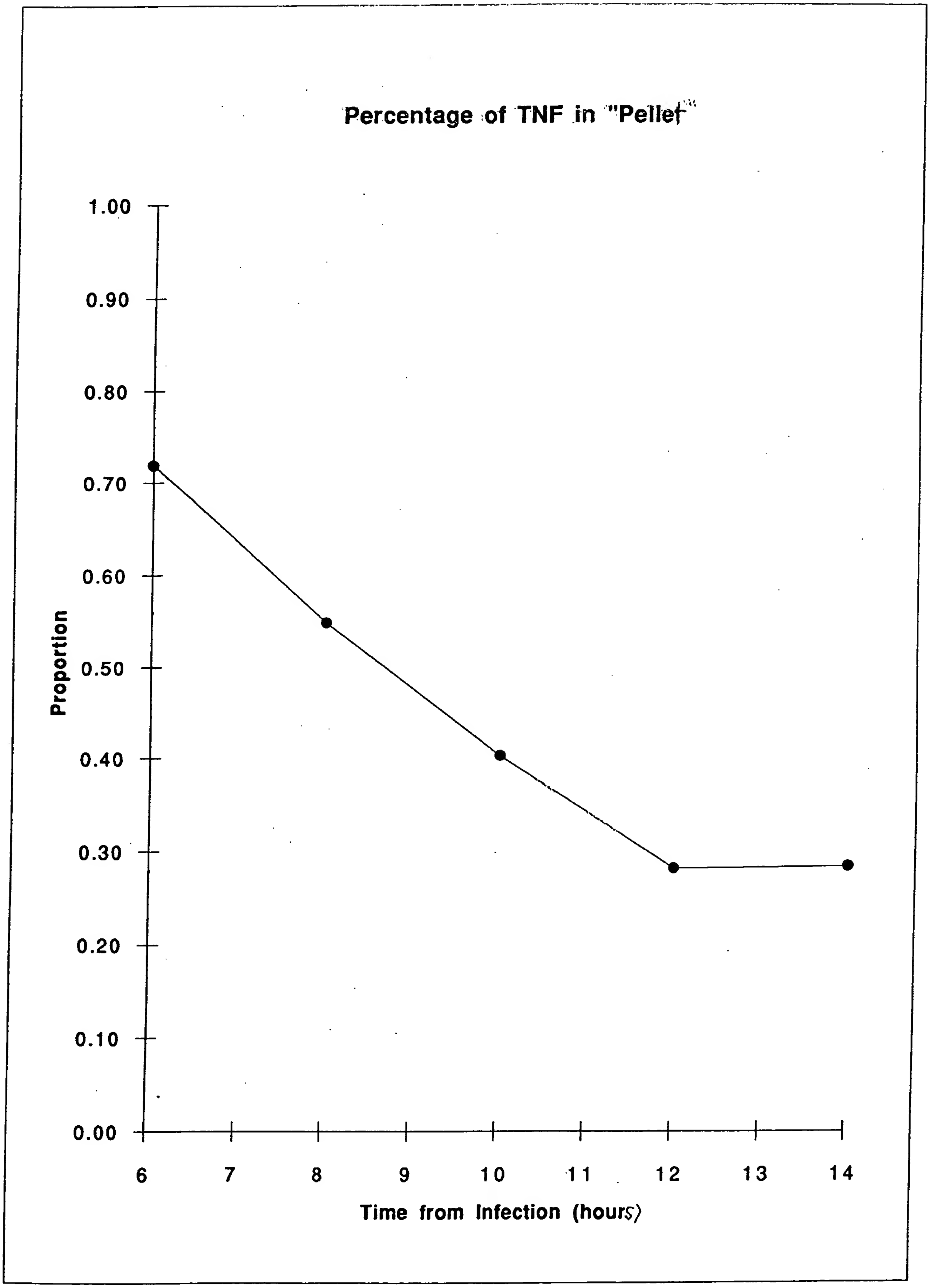
• EXPONENTIAL FIT •
Y= 250.46143*(X)^1.196816
Coef. of Regression= 0.993049 For 6 Data Pairs



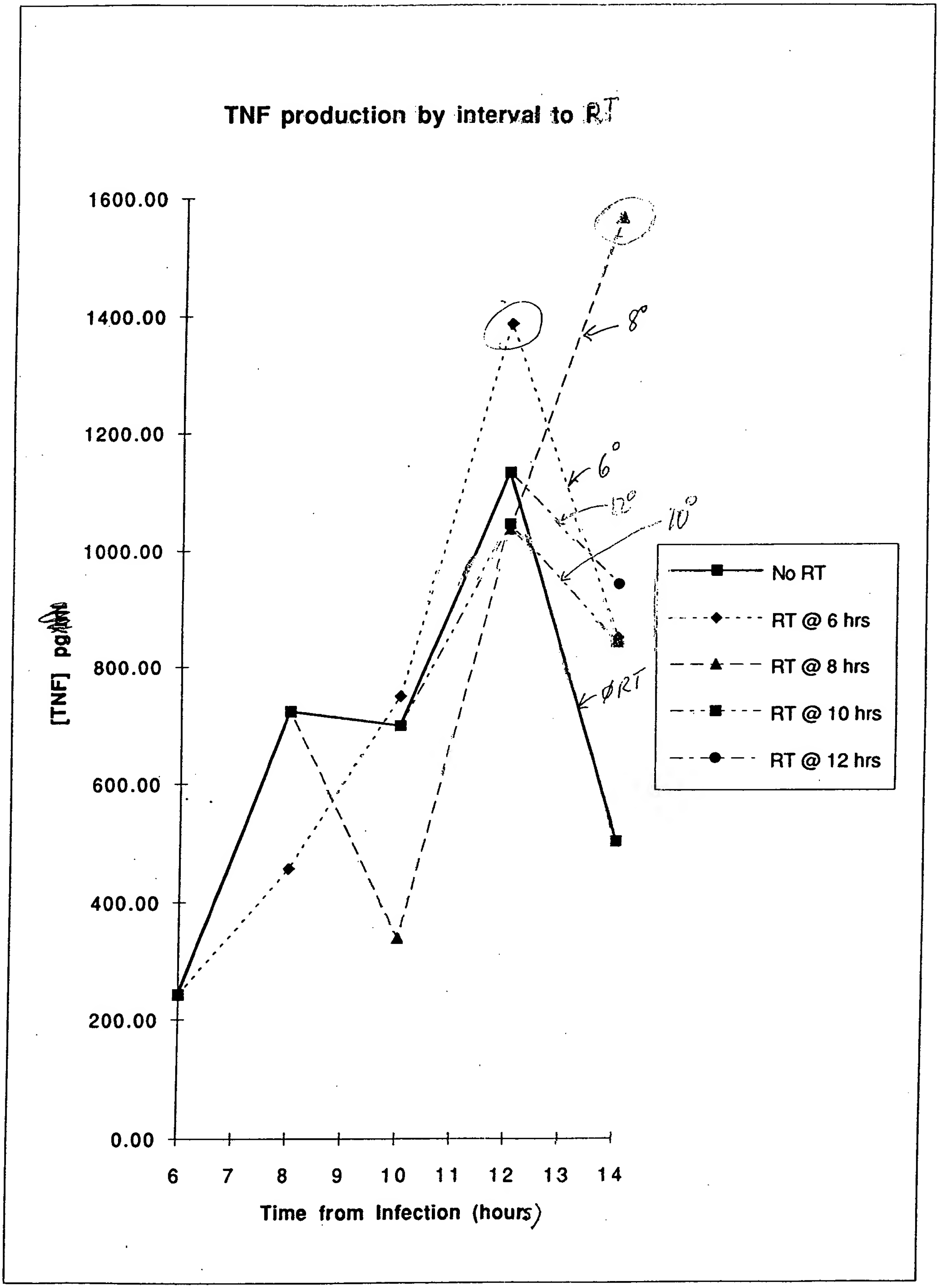
INITIALS GSS SUBJECT • Untitled
DATE
?
CPU Time: 11 Secs.

TNF ELISA **U-87 Cells**

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Total [TNF]
0.107	0.105	0.106	17.07	None	6 hours	supernatant	68.28	243.08
0.225	0.24	0.2325	43.70	None	6 hours	pellet	174.80	
0.389	0.395	0.392	81.66	None	8 hours	supernatant	326.62	723.19
0.46	0.462	0.461	99.14	None	8 hours	pellet	396.57	
0.294	0.299	0.2965	58.46	6 hours	8 hours	supernatant	233.84	456.40
0.281	0.288	0.2845	55.64	6 hours	8 hours	pellet	222.56	
0.484	0.478	0.481	104.31	None	10 hours	supernatant	417.24	699.51
0.349	0.345	0.347	70.57	None	10 hours	pellet	282.27	
0.267	0.262	0.2645	50.99	8 hours	10 hours	supernatant	203.97	339.52
0.188	0.188	0.188	33.89	8 hours	10 hours	pellet	135.55	
0.514	0.534	0.524	115.57	6 hours	10 hours	supernatant	462.27	749.90
0.339	0.366	0.3525	71.91	6 hours	10 hours	pellet	287.63	
0.817	0.865	0.841	203.58	None	12 hours	supernatant	814.32	1134.47
0.379	0.392	0.3855	80.04	None	12 hours	pellet	320.15	
0.755	0.767	0.761	180.63	10 hours	12 hours	supernatant	722.50	1046.13
0.388	0.39	0.389	80.91	10 hours	12 hours	pellet	323.63	
0.709	0.795	0.752	178.07	8 hours	12 hours	supernatant	712.29	1038.41
0.374	0.409	0.3915	81.53	8 hours	12 hours	pellet	326.12	
0.974	1.067	1.0205	256.62	6 hours	12 hours	supernatant	1026.47	1387.28
0.422	0.43	0.426	90.20	6 hours	12 hours	pellet	360.81	
0.431	0.419	0.425	89.95	None	14 hours	supernatant	359.79	503.15
0.196	0.198	0.197	35.84	None	14 hours	pellet	143.36	
0.72	0.737	0.7285	171.43	12 hours	14 hours	supernatant	685.73	943.84
0.316	0.328	0.322	64.53	12 hours	14 hours	pellet	258.11	
0.639	0.655	0.647	148.74	10 hours	14 hours	supernatant	594.96	843.50
0.308	0.316	0.312	62.14	10 hours	14 hours	pellet	248.54	
1.174	1.202	1.188	307.81	8 hours	14 hours	supernatant	1231.23	1566.84
0.399	0.403	0.401	83.90	8 hours	14 hours	pellet	335.62	
0.68	0.661	0.6705	155.23	6 hours	14 hours	supernatant	620.92	852.40
0.284	0.304	0.294	57.87	6 hours	14 hours	pellet	231.48	



Percentage of TNF in "Pellet"
Proportion
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
6
7
8
9
10
11
12
13
14
Time from Infection (hours)



TNF production by interval to RT
1600.00
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
[TNF] pg/ml
6
7
8
9
10
11
12
13
14
Time from Infection (hours)
No RT
RT @ 6 hrs
RT @ 8 hrs
RT @ 10 hrs
RT @ 12 hrs
8°
6°
12°
10°
ØRT

Greg's Mouse Log

Mouse #	Cage #	Tumor Date												
1698	AA104544		12	10	6	345	14	11	10	742.5	15	16.5	10
1720	AA104544		4.5	3.5	3.5	27.563	6.5	6	4.5	87.75	9	9	5.5
1723	AA104544		3.5	3.5	1.5	9.1875	3	3	2.5	11.25	4.5	5.5	2.5
1724	AA104544		4	3.5	1.5	10.5	4	3	2	12	4.5	5	2
1697	AA104549		5	4.5	3.5	39.375	5	4.5	4.5	50.625	6.5	8	5
1702	AA104549		3	3	2	9	4.5	4	5	45	5	5.5	3
1713	AA104549		5	5	3	37.5	6	5.5	5	82.5	9	9	5
1704	AA104550		8.5	6.5	4.5	124.31	9.5	8	6	228	11.5	13.5	9
1717	AA104550		7.3	6.5	4	94.25	6	6.5	5.5	107.25	9.5	8	7
1719	AA104550		6	6	3.5	63	7	7	6	147	8	8.5	5
1722	AA104550		7	6	4.5	94.5	7	6.5	6.5	147.88	9.5	8	4
1707	AA105283		8	5	3.5	70	7	7.5	3	78.75	10.5	10.5	5
1708	AA105283		4	4	2.5	20	3.5	3.5	2.5	15.313	6.5	6	4
1709	AA105283		4	4	2	16	4	3	2	12	6.5	4.5	3
1710	AA105283		5	4	3	30	6.5	6	4	78	9	8.5	5.5

	Mouse #	Cage #	Group	Infect Date	RT Date	Tumor size										wts
283	1720	AA104544	10^9 899-6					6.5	6	4.5	**87.75**	9	9	5.5	**222.75**	25.0
	1710	AA105283	10^9 899-6					6.5	6	4	**78**	9	8.5	5.5	**210.38**	21.7
	1707	AA105283	10^9 899-6					7	7.5	3	**78.75**	11	11	5	**275.63**	20.0
283	1713	AA104549	10^9 899-6					6	5.5	5	**82.5**	9	9	5	**202.5**	23.0
	1698	AA104544						14	11	10	**742.5**	15	17	10	**1237.5**	23.2
	1723	AA104544						3	3	2.5	**11.25**	4.5	5.5	2.5	**30.938**	24.5
	1724	AA104544						4	3	2	**12**	4.5	5	2	**22.5**	25.9
	1697	AA104549						5	4.5	4.5	**50.625**	6.5	8	5	**130**	25.2
	1702	AA104549						4.5	4	5	**45**	5	5.5	3	**41.25**	26.0
	1704	AA104550	Control					9.5	8	6	**228**	12	14	9	**698.63**	24.8
	1717	AA104550	Control					6	6.5	5.5	**107.25**	9.5	8	7	**266**	25.2
	1719	AA104550	Control					7	7	6	**147**	8	8.5	5	**170**	21.4
	1722	AA104550	Control					7	6.5	6.5	**147.88**	9.5	8	4	**152**	24.5
549	1708	AA105283	~~[illegible]~~					3.5	3.5	2.5	**15.313**	6.5	6	4	**78**	23.1
549	1709	AA105283	~~[illegible]~~					4	3	2	**12**	6.5	4.5	3	**43.875**	26.8

THE UNIVERSITY OF CHICAGO
DEPARTMENT OF RADIATION & CELLULAR ONCOLOGY
DIVISION OF THE BIOLOGICAL SCIENCES AND
THE PRITZKER SCHOOL OF MEDICINE

University of Chicago Medical Center
5841 South Maryland Avenue, MC 0085
Chicago, Illinois 60637

Main Office: (312) 702-6819
Appointment Desk: (312) 702-6860
Facsimile: (312) 702-0610

Date:

To: James Linsley
From: ~~Helena J. Mauceri~~ Gregory S. Sibley, MD

Please transfer the cages listed below from CLSC 1053 to Carlson room ~~J-019~~ J-013.
Please have the transfer completed by 5:00 p.m. _______________.
If this is not possible please call me at 2-0492.

Thank you.

Greg Sibley BP #3439
EXT 2-0294

Cage numbers:

~~1859~~

~~AA108958 - AA108964~~ (7 cages)

AA10966 - AA 108972

Mouse # 1859 - 1886

TNF U87 – in vitro

PURPOSE – To determine whether TNF production is ~~Prod~~ induced with 9 Gy RT following R899-6 infection (10^6 PFU = ~ MOI = 0.5) in U87 cells.

METHODS – From previous experiments the optimal interval from infection to RT was 6 hrs. ~~And~~ Optimal interval following RT was 6–8 hrs (total interval 12–14 hrs from infection.)

1. See methods from 2/1/95. Briefly, 10^6 PFU of R899-6 was placed in 60 mm plates of subconfluent (~ 2×10^6 cells) U87 cells & incubate x2° in 1% CV media @ 37°.
2. 10% FCS Media + IgG ~~added~~ @ 2°
3. 9 Gy RT given @ 6° to RT group.
4. Plates sampled at 12 & 14° for "supernatant" & "pellet"

TNF - U87

Sibley / Hyland

$y = 216.779 \times x^{1.154631}$

.0495

1	2	3	4	5	6	7	8	9
1-2	3-4	5-6	7-8	9-10	11-12	13-14		
0	12 S	12 P	12 RT S	14 S	14 P	"		
15	12 S	12 P	12 RT S	"	"	"		
31	12 S	12 P	12 RT P	"	"	14 RT P		
63	12 S	12 P	12 RT P	"	"	"		
125	12 S	12 RT S	12 RT P	"	14 RT S	"		
250	12 S	12 RT S	12 RT P	"	"	"		
500	12 P	12 RT S	12 RT P	14 P	"	"		
1000	12 P	12 RT S	12 RT P	"	"	"		

EFFICIENCY LINE® 22-206 AMPAD

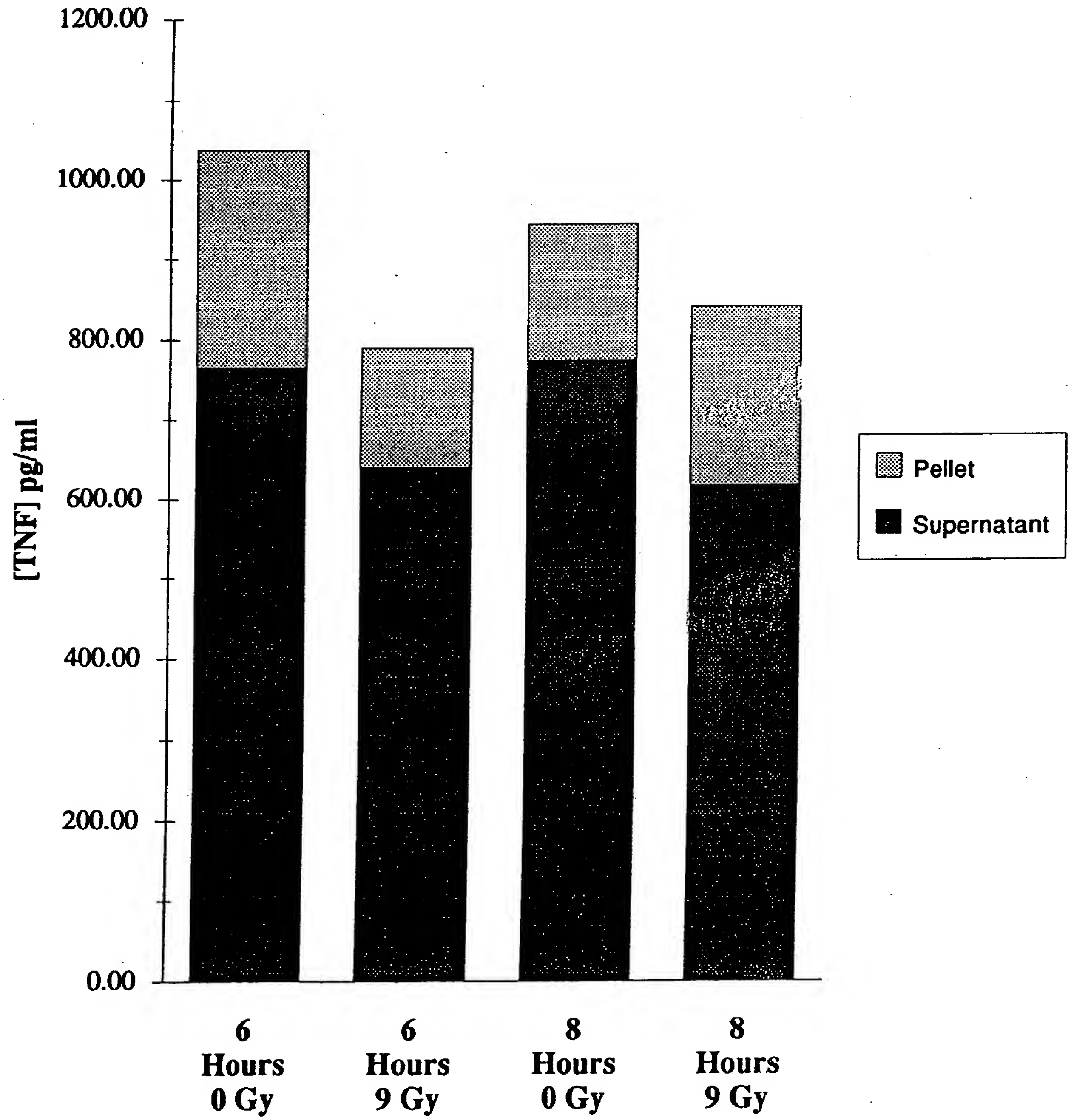
3
14
×8
112
×2
224

TNF ELISA U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Ave [TNF]	Total TNF
0.908	0.896	0.902	192.44	none	12 hours	supernatant	769.76	764.69	1038.11
0.846	0.748	0.797	166.82	none	12 hours	supernatant	667.26		
0.892	0.847	0.8695	184.46	none	12 hours	supernatant	737.83		
0.857	0.842	0.8495	179.57	none	12 hours	supernatant	718.27		
0.936	0.945	0.9405	201.96	none	12 hours	supernatant	807.82		
1.014	1.026	1.02	221.79	none	12 hours	supernatant	887.17		
0.368	0.372	0.37	68.78	none	12 hours	pellet	275.11	273.43	
0.383	0.386	0.3845	71.90	none	12 hours	pellet	287.60		
0.363	0.36	0.3615	66.96	none	12 hours	pellet	267.83		
0.392	0.377	0.3845	71.90	none	12 hours	pellet	287.60		
0.366	0.33	0.348	64.08	none	12 hours	pellet	256.31		
0.368	0.351	0.3595	66.53	none	12 hours	pellet	266.12		
0.824	0.884	0.854	180.67	6 hours	12 hours	supernatant	722.66	640.45	789.94
0.791	0.835	0.813	170.69	6 hours	12 hours	supernatant	682.76		
0.833	0.821	0.827	174.09	6 hours	12 hours	supernatant	696.35		
0.734	0.721	0.7275	150.14	6 hours	12 hours	supernatant	600.54		
0.693	0.8	0.7465	154.67	6 hours	12 hours	supernatant	618.69		
0.602	0.686	0.644	130.42	6 hours	12 hours	supernatant	521.69		
0.234	0.251	0.2425	42.23	6 hours	12 hours	pellet	168.91	149.49	
0.188	0.219	0.2035	34.49	6 hours	12 hours	pellet	137.95		
0.21	0.241	0.2255	38.83	6 hours	12 hours	pellet	155.31		
0.234	0.222	0.228	39.33	6 hours	12 hours	pellet	157.30		
0.209	0.21	0.2095	35.66	6 hours	12 hours	pellet	142.66		
0.203	0.196	0.1995	33.71	6 hours	12 hours	pellet	134.82		
0.876	0.93	0.903	192.69	none	14 hours	supernatant	770.75	774.25	943.63
0.813	0.792	0.8025	168.15	none	14 hours	supernatant	672.58		
0.935	0.947	0.941	202.08	none	14 hours	supernatant	808.32		
0.924	0.9	0.912	194.91	none	14 hours	supernatant	779.63		
0.969	0.953	0.961	207.05	none	14 hours	supernatant	828.19		
0.924	0.913	0.9185	196.51	none	14 hours	supernatant	786.04		
0.248	0.249	0.2485	43.44	none	14 hours	pellet	173.74	169.38	
0.27	0.271	0.2705	47.90	none	14 hours	pellet	191.62		
0.251	0.254	0.2525	44.24	none	14 hours	pellet	176.97		
0.233	0.24	0.2365	41.02	none	14 hours	pellet	164.09		
0.244	0.224	0.234	40.52	none	14 hours	pellet	162.09		
0.211	0.221	0.216	36.95	none	14 hours	pellet	147.78		
0.65	0.653	0.6515	132.18	6 hours	14 hours	supernatant	528.71	616.03	840.68
0.722	0.696	0.709	145.74	6 hours	14 hours	supernatant	582.95		
0.62	0.555	0.5875	117.30	6 hours	14 hours	supernatant	469.21		
0.775	0.784	0.7795	162.59	6 hours	14 hours	supernatant	650.38		

0.886	0.814	0.85	179.69	6 hours	14 hours	supernatant	718.76	
0.895	0.861	0.878	186.54	6 hours	14 hours	supernatant	746.16	
0.314	0.294	0.304	54.82	6 hours	14 hours	pellet	219.27	224.66
0.307	0.303	0.305	55.03	6 hours	14 hours	pellet	220.11	
0.296	0.31	0.303	54.61	6 hours	14 hours	pellet	218.44	
0.295	0.307	0.301	54.19	6 hours	14 hours	pellet	216.78	
0.315	0.312	0.3135	56.80	6 hours	14 hours	pellet	227.20	
0.343	0.329	0.336	61.53	6 hours	14 hours	pellet	246.14	

TNF Production By RT in U87 Cells Following R899-6 Infection



Greg's Mouse Log

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date				Tumvol	weight				Tumvol	weight
1704	AA104550	Control		None	None	14	14.5	13	0	22.1				0	
1717	AA104550	Control		None	None	12.5	11.5	9.5	0	23.9				0	
1719	AA104550	Control		None	None	10.5	12	7	0	21.7				0	
1722	AA104550	Control		None	None	13	14	10	0	25				0	
1720	AA105283	10^7 899-6			None	11	12	8	0	25.1				0	
1710	AA105283	10^7 899-6			None	13	14.5	7.5	0	21.6				0	
1707	AA105283	10^7 899-6			None	15.5	15	8.5	0	18.1				0	
1713	AA105283	10^7 899-6			None	7.5	7.5	3.5	0	23.2				0	
1697	AA104549	10^7 3616			None	11	11	7	0	24.2				0	
1708	AA104549	10^7 3616			None	9.5	9	6	0	24.0				0	
1709	AA104549	10^7 3616			None	9.5	10	6.5	0	27.7				0	
1723	AA104549	10^7 3616			None	8.5	8	5.5	0	25.3				0	
1859	AA108972	RT		None		10	9	6	0	24.4				0	
1860	AA108972	RT		None		13	11.5	7	0	20.2				0	
1861	AA108972	RT		None		9.5	10	6	0	19.1				0	
1862	AA108972	RT		None		12	8.5	7	0	22.9				0	
1702	AA104544					6	6.5	3.5	/ /	26.5					
1724	AA104544					7	5.5	3.5	/ /	24					
1863	AA108971	Large		366+RT		10	7	6.5	21.3						
1864	AA108971	Large				12	7	5.5	21.9						
1865	AA108971	Large				8.5	7	5	22.6						
1866	AA108971	Large		o		9.5	6.5	5.5	23.0						
1867	AA108970	Large		RT Alone		8	~~8~~ 6	5	-	22.7					
1868	AA108970	Large		"		6.5	7	6.5		21.2					
1869	AA108970	Large		"		8.5	7	4.5		22.4					
1870	AA108970	Large		"		9	7	6		22.7					
1871	AA108969	Large 899-6				8	6	5		22.2					
1872	AA108969	Large 11+RT		"		8.5	7	6		20.2					
1873	AA108969	Large		"		6.5	6	6.5		21.4					
1874	AA108969	Large 11		"		10.5	9	6		24.5					

Titers

New samples : Virus used for all in vivo exper.

Virus	Dilution	Count		Titer
3616	10^7	Tm	Tm	
	10^8	23	28	2.6×10^9
899-6	10^7	Tm	Tm	
	10^8	29	47	3.8×10^9

Old samples (used for in vitro exper.)

Virus	Dilution	Count		Titer
3616	10^7	Tm	Tm	
	10^8	45	36	4.1×10^9
899-6	10^7	Tm	Tm	
	10^8	35	26	3.1×10^9

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1704	AA104550	Control	21	21	11	0	21.7				0	
1717	AA104550	Control	13	12.5	10.5	0					0	
1719	AA104550	Control	13	11.5	6	0					0	
1722	AA104550	Control	14	13.5	10	0					0	
1867	AA108970	Control	-10	8	6							
1868	AA108970	Control	-10	9.5	7.5							
1869	AA108970	Control	-10	9	7							
1870	AA108970	Control	-10.5	8.5	8							
		Control		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1720	AA105283	10^7 899-6	12	11	7.5	0					0	
1710	AA105283	10^7 899-6	16	13.5	7	0					0	
1707	AA105283	10^7 899-6	16	15	11	0	17.2				0	
1713	AA105283	10^7 899-6	6.5	5.5	3	0					0	
		10^7 899-6		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1697	AA104549	10^7 3616	-10	9	7	0					0	
1708	AA104549	10^7 3616	8	8.5	5	0					0	
1709	AA104549	10^7 3616	9	10	7	0					0	
1723	AA104549	10^7 3616	7.5	9	6	0					0	
		10^7 3616		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1859	AA108972	RT Alone	9.5	9.5	5.5	0					0	
1860	AA108972	RT Alone	12	10.5	8	0					0	
1861	AA108972	RT Alone	10.5	9	7	0					0	
1862	AA108972	RT Alone	12	9	7	0					0	
		RT Alone		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	

1863	AA108971	10^7 3616+RT	9	8	6.5	0					0	
1864	AA108971	10^7 3616+RT	12.5	7.5	6	0					0	
1865	AA108971	10^7 3616+RT	10	9	6.5	0					0	
1866	AA108971	10^7 3616+RT	10.5	7.5	5.5	0					0	
1883	AA108966	10^7 3616+RT	7.5	7	5	0					0	
1884	AA108966	10^7 3616+RT	11.5	10	8	0					0	
1885	AA108966	10^7 3616+RT	12	9	7	0					0	
1886	AA108966	10^7 3616+RT	7.5	7.5	5	0					0	
		10^7 3616+RT		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		0			Day:		0	
1871	AA108969	10^7 899-6+RT	9	8	5.5	0					0	
1872	AA108969	10^7 899-6+RT	9.5	7.5	6.5	0					0	
1873	AA108969	10^7 899-6+RT	9.5	8	7	0					0	
1874	AA108969	10^7 899-6+RT	11	10.5	8	0					0	
1879	AA108967	10^7 899-6+RT	8	9	6	0					0	
1880	AA108967	10^7 899-6+RT	8	7	6	0					0	
1881	AA108967	10^7 899-6+RT	8	7	5	0					0	
1882	AA108967	10^7 899-6+RT	9.5	8	5	0					0	
		10^7 899-6+RT		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		0			Day:		0	
1887	AA108965	10^7 899-6	8.5	6	5		22.5					
1888	AA108965	"	8.5	5.5	4.5		23.4					
1889	AA108965	"	5.5	8	5		22.8					
1890	AA108965	"	7.5	6.5	5		23.3					
				Mean:		#DIV/0!			Mean:		#DIV/0!	
				Std Error:		#DIV/0!			Std Error:		#DIV/0!	
				Day:		0			Day:		0	
1875	AA108968											
1876	AA108968											
1877	AA108968											
1878	AA108968											
				Mean:		#DIV/0!			Mean:		#DIV/0!	

				Std Error:		#DIV/0!			Std Error:		#DIV/0!	
				Day:		0			Day:		0	
1891	AA108964											
1892	AA108964											
1893	AA108964											
1894	AA108964											
1895	AA108963											
1896	AA108963											
1897	AA108963											
1898	AA108963											
1899	AA108962											
1900	AA108962											
1901	AA108962											
1902	AA108962											
1903	AA108961											
1904	AA108961											
1905	AA108961											
1906	AA108961											
1907	AA108960											
1908	AA108960											
1909	AA108960											
1910	AA108960											
1911	AA108959											
1912	AA108959											
1913	AA108959											
1914	AA108959											
1915	AA108958											
1916	AA108958											
1917	AA108958											
1918	AA108958											
1702	AA104544											
1724	AA104544											

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 18781

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ____ SHIPMENTS ON A ____ BASIS

REQUEST BY: Greg Sibley, MD DATE:

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE:

FAS ACCOUNT: 2-7371-5100

VENDOR: FCRI

P.I.: Hallahan

PROTOCOL: 58671

PHONE: 2-0294

REQUESTED DELIVERY DATE:

SPECIES: Mouse

QUANTITY: 60

STRAIN: Athymic Nude

SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

HOUSE AT: ____ CARLSON ____ WYLER X CLSC ____ FMI ____ OTHER

The University of Chicago Departmental Purchase Order

Greg

Z 873342
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
USA Orders - Cust #112486
Life Technologies, Inc
P.O. Box 68
Grand Island | NY | 14072
City | State | Zip Code
Payment Terms ____ Delivery charge? ☐ Yes ☒ No
Telephone No. 1-800-828-6686 FAX No. 1-800-331-2286

Ship To
THE UNIVERSITY OF CHICAGO — Dept. Code: ____
Department: Radiation Oncology MC-0085
Attn: Greg Sibley, MD — Room: G-03
5830 S. Ellis Ave
City: Chicago | State: IL | Zip Code: 60637

372-369

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services that should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ____ Payroll No. ____
Print Name ____ Ext. ____
Account Code ____ Date ____

Order placed by phone? ☐ No ☐ Yes
Order placed with (name): Melissa Ref #372369 Date ____
Order placed by (name): Greg Sibley, MD Phone # ____

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
24		11095-023 Minimal Essential Media 500 ml	8.64	207.36
12 ~~25~~		15140-015 Penicillin - Streptomycin 100 ml	10.00	~~250.00~~ 120.00
~~18~~		~~22400-055 RPMI 1640 500 ml~~		
12		22400-055 RPMI 1640 Media 500 ml	12.91	154.92
			ORDER TOTAL	482.28

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00

BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
~~1704~~	~~AA104550~~	~~Control~~				0					0	
1717	AA104550	Control.	18	15.5	14	0					0	
1719	AA104550	Control	12	15	8	0					0	
1722	AA104550	Control	19.5	18	13	0					0	
1867	AA108970	Control	13	16	9.5							
1868	AA108970	Control	18	15	13							
1869	AA108970	Control	15.5	16	9							
1870	AA108970	Control	15.5	13	11							
		Control		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1720	AA105283	10^7 899-6	11.5	11.5	8	0					0	
1710	AA105283	10^7 899-6	19.5	16	8	0					0	
1707	AA105283	10^7 899-6	19	16.5	13	0					0	
1713	AA105283	10^7 899-6	Ø			0					0	
		10^7 899-6				0					0	
				Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1697	AA104549	10^7 3616	11	8.5	5.5	0					0	
1708	AA104549	10^7 3616	5.5	7	3.5	0					0	
1709	AA104549	10^7 3616	6	7.5	4	0					0	
1723	AA104549	10^7 3616	8	9	5	0					0	
		10^7 3616									0	
				Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1859	AA108972	RT Alone	10	11.5	6.5	0					0	
1860	AA108972	RT Alone	9	8	6.5	0					0	
1861	AA108972	RT Alone	11.5	12.5	8	0					0	
1862	AA108972	RT Alone	11	9	4	0					0	
		RT Alone									0	
				Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	

1863	AA108971	10^7 3616+RT	11.5	9	6	0				0	
1864	AA108971	10^7 3616+RT	12.5	8	4.5	0				0	
1865	AA108971	10^7 3616+RT	6.5	8	5	0				0	
1866	AA108971	10^7 3616+RT	8.5	8	5.5	0				0	
1883	AA108966	10^7 3616+RT	6	5.5	4	0				0	
1884	AA108966	10^7 3616+RT	12.5	10.5	7	0				0	
1885	AA108966	10^7 3616+RT	11.5	9.5	5	0				0	
1886	AA108966	10^7 3616+RT	5.5	5	4	0				0	
		10^7 3616+RT		Mean:		0			Mean:	0	
				Std Error:		0			Std Error:	0	
				Day:		0			Day:	0	
	96										
1871	AA108969	10^7 899-6+RT	8	7.5	5.5	0				0	
1872	AA108969	10^7 899-6+RT	8	6.5	5	0				0	
1873	AA108969	10^7 899-6+RT	8.5	6.5	5	0				0	
1874	AA108969	10^7 899-6+RT	9	9	6	0				0	
1879	AA108967	10^7 899-6+RT	5	4.5	3.5	0				0	
1880	AA108967	10^7 899-6+RT	5.6	4.5	3	0				0	
1881	AA108967	10^7 899-6+RT	7	7	3	0				0	
1882	AA108967	10^7 899-6+RT	7	5	3	0				0	
		10^7 899-6+RT		Mean:		0			Mean:	0	
				Std Error:		0			Std Error:	0	
				Day:		0			Day:	0	
1887	AA108965		8.5	8.5	5						
1888	AA108965		8	7.5	6						
1889	AA108965		5	5.5	3						
1890	AA108965		8	8	4						
				Mean:		#DIV/0!			Mean:	#DIV/0!	
				Std Error:		#DIV/0!			Std Error:	#DIV/0!	
				Day:		0			Day:	0	
1875	AA108968		13	11.5	8						
1876	AA108968		10	9	7.5						
1877	AA108968		10	9.5	8						
1878	AA108968		8.5	8	5.5						
				Mean:		#DIV/0!			Mean:	#DIV,	

12.5 10.5 7

8.5 6.5 5

				Std Error:		#DIV/0!			Std Error:		#DIV/0!	
				Day:		0			Day:		0	
1891	AA108964	Rt Above	6.5	7	5.5							
1892	AA108964	"	8	9	5							
1893	AA108964	"	9.5	7.5	7							
1894	AA108964	"	8	8.5	6							
1895	AA108963											
1896	AA108963											
1897	AA108963											
1898	AA108963											
1899	AA108962											
1900	AA108962											
1901	AA108962											
1902	AA108962											
1903	AA108961											
1904	AA108961											
1905	AA108961											
1906	AA108961											
1907	AA108960											
1908	AA108960											
1909	AA108960											
1910	AA108960											
1911	AA108959											
1912	AA108959											
1913	AA108959											
1914	AA108959											
1915	AA108958											
1916	AA108958											
1917	AA108958											
1918	AA108958											
1702	AA104544											
1724	AA104544											

Jim Linsley
P-110
Animal Resources

Dear Jim,

Please transfer the cages listed below from CLSC 1053 to Carlson room **J-O13**.
Please have the transfer completed by 5 pm on

If this is not possible, please contact me.

Thank you,

Greg Sibley, M.D.
Dept. of Radiation and Cellular Oncology
Beeper #3439
Extension 2-0294

Cage Numbers:
AA111326 through AA111333

Total Number of Cages: **8**

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6	17.5	12	9.5							
1710	AA105283	10^7 899-6	6.5	6.5	1.5							
1713	AA105283	10^7 899-6	0	0	0							
		10^7 899-6										
1697	AA104549	10^7 3616	15.5	13.5	9							
1708	AA104549	10^7 3616	2	2.5	1.5							
1709	AA104549	10^7 3616	5	5	1							
1723	AA104549	10^7 3616	0	0	0							
1875	AA108968	10^7 3616	19	18.5	10							
1876	AA108968	10^7 3616	18	17	11.5							
1877	AA108968	10^7 3616	18.5	14	13							
1878	AA108968	10^7 3616	16	12.5	11							
		10^7 3616										
1859	AA108972	RT Alone	7	1.5	4							
1860	AA108972	RT Alone 13×11×6	~~[illegible]~~	~~7.5~~	~~5~~							
1861	AA108972	RT Alone	10	10.5	7							
1862	AA108972	RT Alone	8.5	7.5	3							
1891	AA108964	RT Alone	6.5	6.5	5							
1892	AA108964	RT Alone	12.5	9.5	6							
1893	AA108964	RT Alone	10	9.5	5.5							
1894	AA108964	RT Alone	8	8	5							
		RT Alone										
1863	AA108971	10^7 3616+RT	10	8	4							
1864	AA108971	10^7 3616+RT	10.5	7	4							
1865	AA108971	10^7 3616+RT	5.5	6.5	4.5							
1866	AA108971	10^7 3616+RT	7	5	4.5							
1883	AA108966	10^7 3616+RT	3.5	3	1.5							
1884	AA108966	10^7 3616+RT	5	9	6							
1885	AA108966	10^7 3616+RT	7	8.5	4							
1886	AA108966	10^7 3616+RT	3.5	4	2							
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT	7.5	7	4.5							
1872	AA108969	10^7 899-6+RT	7	5.5	4.5							
1873	AA108969	10^7 899-6+RT	8	7.5	4.5							
1874	AA108969	10^7 899-6+RT	8	7.5	4							
1879	AA108967	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	3.5	3	1							
1881	AA108967	10^7 899-6+RT	5.5	6	3							
1882	AA108967	10^7 899-6+RT	4	3	1							
		10^7 899-6+RT										
2032	AA111326	10^7 899-6										
2033	AA111326	10^7 899-6										
2034	AA111326	10^7 899-6										
2035	AA111326	10^7 899-6										
2036	AA111327	10^7 899-6										
2037	AA111327	10^7 899-6										
2038	AA111327	10^7 899-6										
2039	AA111327	10^7 899-6										
2040	AA111328	10^7 899-6										
2041	AA111328	10^7 899-6										
2042	AA111328	10^7 899-6										
2043	AA111328	10^7 899-6										
		10^7 899-6										
2044	AA111329	10^7 3616										
2045	AA111329	10^7 3616										
2046	AA111329	10^7 3616										
2047	AA111329	10^7 3616										
2048	AA111330	10^7 3616										
2049	AA111330	10^7 3616										
2050	AA111330	10^7 3616										
2051	AA111330	10^7 3616										
		10^7 3616										
2052	AA111331	10^7 899-6+RT										
2053	AA111331	10^7 899-6+RT										
2054	AA111331	10^7 899-6+RT										
2055	AA111331	10^7 899-6+RT										
2056	AA111332	10^7 899-6+R										
2057	AA111332	10^7 899-										
2058	AA111332	10^7 899										
2059	AA111332	10^7 899										
		10^7 899-6+R										

2060	AA111333	10^7 3616+RT										
2061	AA111333	10^7 3616+RT										
2062	AA111333	10^7 3616+RT										
2063	AA111333	10^7 3616+RT										
2067	AA111335	10^7 3616+RT										
2068	AA111335	10^7 3616+RT										
2069	AA111335	10^7 3616+RT										
2070	AA111335	10^7 3616+RT										
		10^7 3616+RT										

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6										
1710	AA105283	10^7 899-6										
1713	AA105283	10^7 899-6										
		10^7 899-6										
1697	AA104549	10^7 3616										
1708	AA104549	10^7 3616										
1709	AA104549	10^7 3616										
1723	AA104549	10^7 3616										
1875	AA108968	10^7 3616										
1876	AA108968	10^7 3616										
1877	AA108968	10^7 3616										
1878	AA108968	10^7 3616										
		10^7 3616										
1859	AA108972	RT Alone										
1860	AA108972	RT Alone										
1861	AA108972	RT Alone										
1862	AA108972	RT Alone										
1891	AA108964	RT Alone										
1892	AA108964	RT Alone										
1893	AA108964	RT Alone										
1894	AA108964	RT Alone										
		RT Alone										
1863	AA108971	10^7 3616+RT										
1864	AA108971	10^7 3616+RT										
1865	AA108971	10^7 3616+RT										
1866	AA108971	10^7 3616+RT										
1883	AA108966	10^7 3616+RT										
1884	AA108966	10^7 3616+RT										
1885	AA108966	10^7 3616+RT										
1886	AA108966	10^7 3616+RT										
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT										
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT										
1874	AA108969	10^7 899-6+RT										
1879	AA108967	10^7 899-6+RT										
1880	AA108967	10^7 899-6+RT										
1881	AA108967	10^7 899-6+RT										
1882	AA108967	10^7 899-6+RT										
		10^7 899-6+RT										
2032	AA111326	10^7 899-6 ~~+RT~~	9	7	6		22.2	3616	+RT			
2033	AA111326	10^7 899-6 vm	11	7.5	6.5		22.6	"				
2034	AA111326	10^7 899-6 vm	11	10	7		19.6	"				
2035	AA111326	10^7 899-6 vm	12.5	7	6		19.0	"				
2036	AA111327	10^7 899-6 ~~+RT~~	10	7	5		19.7	3616	+RT			
2037	AA111327	10^7 899-6 vm	8	7	4.5		21.7	"				
2038	AA111327	10^7 899-6 vm	11.5	9.5	7.5		13.0	"				
2039	AA111327	10^7 899-6 vm	9.5	7	5		[illegible]	"				
2040	AA111328	10^7 899-6	11	9.5	7		21.3	3616				
2041	AA111328	10^7 899-6	6.5	6	5		21.3	~~899-6~~	~~+RT~~	~~"~~		
2042	AA111328	10^7 899-6	10	10	7.5		23.2	"				
2043	AA111328	10^7 899-6	8	6.5	5.5		23.7	"				
		10^7 899-6										
2044	AA111329	10^7 3616+RT	8.5	7	4.5		19.3	899-6	+RT			
2045	AA111329	10^7 3616 +RT	7	6	4		22.4	"				
2046	AA111329	10^7 3616 "	6.5	6	5		[illegible]	"				
2047	AA111329	10^7 3616 "	9	8.5	5		[illegible]	"				
2048	AA111330	10^7 3616 +RT	8	5	4.5		19.2	899-6	+RT			
2049	AA111330	10^7 3616 "	11	7	5.5		22.6	"				
2050	AA111330	10^7 3616 "	8.5	6.5	5.5		21.1	"				
2051	AA111330	10^7 3616 "	8.5	8.5	5.5		19.2	"				
		10^7 3616										
2052	AA111331	10^7 899-6+RT	7	6	5		[illegible]	899-6				
2053	AA111331	10^7 899-6+RT	7	6	4.5		[illegible]	899-6				
2054	AA111331	10^7 899-6+RT	7	6	4.5		[illegible]	"				
2055	AA111331	10^7 899-6+RT	"	6.5	5		[illegible]	"				
2056	AA111332	10^7 899-6+RT	8	5	4		[illegible]	899-6				
2057	AA111332	10^7 899-6+RT	7	6	4.5		21.9	"				
2058	AA111332	10^7 899-6+RT	7	9	5.5		22.9	"				
2059	AA111332	10^7 899-6+RT						"				
		10^7 899-6+RT										

extra injected

Bubba tumors

1331 1 tumor

1332 2 t[illegible]

[illegible]

2060	AA111333	10^7 3616+RT										
2061	AA111333	10^7 3616+RT										
2062	AA111333	10^7 3616+RT										
2063	AA111333	10^7 3616+RT										
2067	AA111335	10^7 3616+RT										
2068	AA111335	10^7 3616+RT										
2069	AA111335	10^7 3616+RT										
2070	AA111335	10^7 3616+RT										
		10^7 3616+RT										

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
720	AA105283	10^7 899-6	+ 20.5	10	13	26.0						
1710	AA105283	10^7 899-6	+ 0	0	0	23.0						
1713	AA105283	10^7 899-6	+ 0	0	0	21.6						
		10^7 899-6										
1697	AA104549	10^7 3616	+ 17.5	14.5	11	23.8						
1708	AA104549	10^7 3616	+ 0	0	0	24.0						
1709	AA104549	10^7 3616	+ 3.5	4	1	28.2						
1723	AA104549	10^7 3616	+ 0	0	0	26.3						
1875	AA108968	10^7 3616	+ 19	20	10	22.3						
1876	AA108968	10^7 3616	+ 17	17	13.5	23.5						
1877	AA108968	10^7 3616	+ 22.5	17.5	16.5	27.3						
1878	AA108968	10^7 3616	+ 18.5	15.5	15.5	26.0						
		10^7 3616										
			10	10	5		25.1					
1859	AA108972	RT Alone	~~9~~	~~12~~	~~8.5~~		~~15.9~~					
1860	AA108972	RT Alone	13	1	8.5		8.7					
1861	AA108972	RT Alone	11	1 5	7		20.9					
862	AA108972	RT Alone	9	7.5	3.5		24.7					
891	AA108964	RT Alone	6.5	7.5	3.5		19.8					
1892	AA108964	RT Alone	7.5	9 5	3		21.5					
1893	AA108964	RT Alone	10	9	5.5		20.8					
1894	AA108964	RT Alone	7.5	8	4		21.5					
		RT Alone										
1863	AA108971	10^7 3616+RT	+ 9.5	8	3.5	22.9						
1864	AA108971	10^7 3616+RT	+ 6	5	4	25.3						
1865	AA108971	10^7 3616+RT	10	6.5	4	24.9						
1866	AA108971	10^7 3616+RT	+ 5	4	3.5	25.1						
1883	AA108966	10^7 3616+RT	+ 4	3	1	21.7						
1884	AA108966	10^7 3616+RT	+ 5.5	7	4.5	23.5						
1885	AA108966	10^7 3616+RT	Dead	3/13/95								
1886	AA108966	10^7 3616+RT	+ 3	4	2	22.5						
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT	8	7	5	24.3						
1872	AA108969	10^7 899-6+RT	12	5.5	4	22.3						
1873	AA108969	10^7 899-6+RT	8	7.5	4.5	23.3						
1874	AA108969	10^7 899-6+RT	3	3	4	26.1						
1879	AA108967	10^7 899-6+RT	0	0	0	25.0						
1880	AA108967	10^7 899-6+RT	0	0	0	21.3						
1881	AA108967	10^7 899-6+RT	5.5	5.5	2.5	24.0						
1882	AA108967	10^7 899-6+RT	4	3	1	22.5						
		10^7 899-6+RT										
2032	AA111326	10^7 899-6										
2033	AA111326	10^7 899-6										
2034	AA111326	10^7 899-6										
2035	AA111326	10^7 899-6										
2036	AA111327	10^7 899-6										
2037	AA111327	10^7 899-6										
2038	AA111327	10^7 899-6										
2039	AA111327	10^7 899-6										
2040	AA111328	10^7 899-6										
2041	AA111328	10^7 899-6										
2042	AA111328	10^7 899-6										
2043	AA111328	10^7 899-6										
		10^7 899-6										
2044	AA111329	10^7 3616										
2045	AA111329	10^7 3616										
2046	AA111329	10^7 3616										
2047	AA111329	10^7 3616										
2048	AA111330	10^7 3616										
2049	AA111330	10^7 3616										
2050	AA111330	10^7 3616										
2051	AA111330	10^7 3616										
		10^7 3616										
2052	AA111331	10^7 899-6+RT										
2053	AA111331	10^7 899-6+RT										
2054	AA111331	10^7 899-6+RT										
2055	AA111331	10^7 899-6+RT										
205[illegible]	AA111332	10^7 899-6+RT										
2[illegible]	AA111332	10^7 899-6+RT										
[illegible]	AA111332	10^7 899-6+RT										
[illegible]	AA111332	10^7 899-6+RT										
		10^7 899-6+RT										

2060	AA111333	10^7 3616+RT										
2061	AA111333	10^7 3616+RT										
2062	AA111333	10^7 3616+RT										
2063	AA111333	10^7 3616+RT										
2067	AA111335	10^7 3616+RT										
2068	AA111335	10^7 3616+RT										
2069	AA111335	10^7 3616+RT										
2070	AA111335	10^7 3616+RT										
		10^7 3616+RT										

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6	21	15	11							
1710	AA105283	10^7 899-6	0	0	0							
1713	AA105283	10^7 899-6	0	0	0							
		10^7 899-6										
1697	AA104549	10^7 3616	18	16	10							
1708	AA104549	10^7 3616	0	0	0							
1709	AA104549	10^7 3616	3.5	3.5	1							
1723	AA104549	10^7 3616	2	2	1							
1875	AA108968	10^7 3616	17.5	19	8							
1876	AA108968	10^7 3616	20	20.5	13.5							
1877	AA108968	10^7 3616	24	18	17							
1878	AA108968	10^7 3616	20.5	17.5	13.5							
		10^7 3616										
1859	AA108972	RT Alone	9.5	10	3.5							
1860	AA108972	RT Alone	13	12.5	5.5							
1861	AA108972	RT Alone	11.5	12	5.5							
1862	AA108972	RT Alone	7	7	4							
1891	AA108964	RT Alone	6	6.5	3.5							
1892	AA108964	RT Alone	12.5	9	6							
1893	AA108964	RT Alone	9.5	8	4.5							
1894	AA108964	RT Alone	7	7	3.5							
		RT Alone										
1863	AA108971	10^7 3616+RT	9.5	8	3							
1864	AA108971	10^7 3616+RT	9	6.5	2							
1865	AA108971	10^7 3616+RT	5	3.5	3							
1866	AA108971	10^7 3616+RT	3	3	1							
1883	AA108966	10^7 3616+RT	3.5	2	1							
1884	AA108966	10^7 3616+RT	8.5	7	5.5							
1885	AA108966	10^7 3616+RT	~~2.5~~	~~3~~								
1886	AA108966	10^7 3616+RT	2.5	3	1							
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT	6	6	3.5							
1872	AA108969	10^7 899-6+RT	5.5	5	3							
1873	AA108969	10^7 899-6+RT	7	7	3.5							
1874	AA108969	10^7 899-6+RT	7.5	7.5	3							
1879	AA108967	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0							
1881	AA108967	10^7 899-6+RT	5.8	6	2.5							
1882	AA108967	10^7 899-6+RT	3	2.5	1							
		10^7 899-6+RT										
2032	AA111326	10^7 899-6	11	8	6							
2033	AA111326	10^7 899-6	13	9	6							
2034	AA111326	10^7 899-6	11.5	11	7							
2035	AA111326	10^7 899-6	12.5	8.5	8							
rets 2036	AA111327	10^7 899-6	11.5	9.5	6							
2037	AA111327	10^7 899-6	9.5	8	5.5							
2038	AA111327	10^7 899-6	13	11	8.5							
2039	AA111327	10^7 899-6	10.5	8.5	6							
2040	AA111328	10^7 899-6	13	13	8							
2041	AA111328	10^7 899-6	8	7.5	5.5							
2042	AA111328	10^7 899-6	12	14	11							
2043	AA111328	10^7 899-6	11	8.5	6							
		10^7 899-6										
2044	AA111329	10^7 3616	11	9	7							
2045	AA111329	10^7 3616	8	6.5	4.5							
2046	AA111329	10^7 3616	9	7.5	6							
2047	AA111329	10^7 3616	10.5	10.5	5							
2048	AA111330	10^7 3616	9	6	4.5							
2049	AA111330	10^7 3616	15	8	6.5							
2050	AA111330	10^7 3616	9	7.5	5.5							
2051	AA111330	10^7 3616	11	10	5.5							
		10^7 3616										
2052	AA111331	10^7 899-6+RT	10	7.5	6.5							
2053	AA111331	10^7 899-6+RT	6.5	8	6							
2054	AA111331	10^7 899-6+RT	9.5	7.5	4.5							
2055	AA111331	10^7 899-6+RT	14	7.5	6.5							
2056	AA111332	10^7 899-6+RT	10	7	4.5							
2057	AA111332	10^7 899-6+RT	7.5	7	5							
2058	AA111332	10^7 899-6+RT	10	12	5							
2059	AA111332	10^7 899-6+RT										
		10^7 899-6+RT										

2060	AA111333	10^7 3616+RT	9.5	6.5	4		21.6	57-6				
2061	AA111333	10^7 3616+RT	8	7	5.5		24.0					
2062	AA111333	10^7 3616+RT	6	7.5	4.5		15.4					
2063	AA111333	10^7 3616+RT	8	7	4.5		21.0					
2067	AA111335	10^7 3616+RT	7.5	6	5		21.3	5616				
2068	AA111335	10^7 3616+RT	10.5	8	6		21.0					
2069	AA111335	10^7 3616+RT	8	7.5	5.5		21.1					
2070	AA111335	10^7 3616+RT	10.5	6	5		17.8					
		10^7 3616+RT										

Greg's Mouse Log

	Mouse #	Cage #	Group	Day 0				weight	Day 3					weight
	1720	AA105283	10^7 899-6	23	10	14		25.2						
	1710	AA105283	10^7 899-6	0	0	0		23.3						
	1713	AA105283	10^7 899-6	0	0	0		22.1						
	2052	AA111331	10^7 899-6	11	9.5	6		18.4						
	2053	AA111331	10^7 899-6	8	10.5	7		21.1						
	2054	AA111331	10^7 899-6	7	6	4.5		19.5						
14	2055	AA111331	10^7 899-6	10	12	7.5		17.4						
	2056	AA111332	10^7 899-6	9	7	5		23.4						
	2057	AA111332	10^7 899-6	7.5	6	4.5		21.8						
2	2058	AA111332	10^7 899-6	11	7.5	5.5		23.5						
	2060	AA111333	10^7 899-6	11	8.5	7		22.3						
	2061	AA111333	10^7 899-6	10	9	7		23.3						
	2062	AA111333	10^7 899-6	9	7.5	6.5		18.0						
	2063	AA111333	10^7 899-6	9.5	7.5	5.5		20.5						
			10^7 899-6											
	1697	AA104549	10^7 3616	18	15	11.5		23.4						
	1708	AA104549	10^7 3616	5	3	1		22.5						
SC	1709	AA104549	10^7 3616	3	3	1		27.6						
	1723	AA104549	10^7 3616	0	0	0		26.1						
	~~1875~~	AA108968	10^7 3616	20.5	16.5	9		15.3	→ SAC					
	1876	AA108968	10^7 3616	Dead										
	1877	AA108968	10^7 3616	26	19	13		26.3						
	1878	AA108968	10^7 3616	22	13.5	11		23.8						
15	~~2040~~	AA111328	10^7 3616	16	14	8.5		22.2	→ AA 111335					
	2041	AA111328	10^7 3616	6	5.5	4		22.2						
12	~~2042~~	AA111328	10^7 3616	17	17	12		23.6	→	"				
	2043	AA111328	10^7 3616	10.5	9	5		24.0						
4	2064	AA111334	10^7 3616	9.5	7	7		21.4						
	2065	AA111334	10^7 3616	~~8~~13	~~8~~13	10		21.1						
	2066	AA111334	10^7 3616	11	7.5	6		16.5						
	2067	AA111334	10^7 3616	~~6~~11	9.5	8		22.2						
			10^7 3616											
	2070			10.5	7	6		22.3						
	1859	AA108972	RT Alone	14.5	14	11		18.5						
	1860	AA108972	RT Alone	8	10	5.5		24.0						
	1861	AA108972	RT Alone	11.5	12	7.5		21.0						
	1862	AA108972	RT Alone	8	6.5	3.5		23.5						
	1891	AA108964	RT Alone	Dead										
	1892	AA108964	RT Alone	8	8	5	water	19.2						
	1893	AA108964	RT Alone	Dead										
	1894	AA108964	RT Alone	Dead										
			RT Alone											

	1863	AA108971	10^7 3616+RT	9	7	3		21.9					
	1864	AA108971	10^7 3616+RT	9	4.5	2.5		23.4					
	1865	AA108971	10^7 3616+RT	3.5	4.5	3		24.6					
	1866	AA108971	10^7 3616+RT	4	3	2		24.[illegible]					
	1883	AA108966	10^7 3616+RT	7	5	1.5		21.8					
	1884	AA108966	10^7 3616+RT	7	6.5	5		21.5					
	1886	AA108966	10^7 3616+RT	4	3	1		21.7					
15	2032	AA111326	10^7 3616+RT	10	6	4.5		20.9					
	2033	AA111326	10^7 3616+RT	11	8	5.5		22.2					
	~~2034~~	AA111326	10^7 3616+RT	14.5	13.5	10		18.2	→ 111 235				
13	2035	AA111326	10^7 3616+RT	12	9	7.5		19.3					
	2036	AA111327	10^7 3616+RT	11	9	5.5		19.4					
3	2037	AA111327	10^7 3616+RT	9	7	5.5		22.9					
	~~2038~~	AA111327	10^7 3616+RT	13	11.5	7.5		17.4	→ 111335				
	2039	AA111327	10^7 3616+RT	9.5	8.5	6		19.0					
			10^7 3616+RT										
	1871	AA108969	10^7 899-6+RT	5	6	4	5	22.7					
	1872	AA108969	10^7 899-6+RT	7	6.5	3	2	23.1					
	1873	AA108969	10^7 899-6+RT	8.5	7	5		22.2					
	1874	AA108969	10^7 899-6+RT	7	6.5	3.5		23.3					
	1879	AA108967	10^7 899-6+RT	0	0	0		25.2					
	1880	AA108967	10^7 899-6+RT	4	4.5	1		21.5					
	1881	AA108967	10^7 899-6+RT	5	6.5	3		23.8					
	1882	AA108967	10^7 899-6+RT	4	3	1		22.0					
	2044	AA111329	10^7 899-6+RT	10	8	6		23.3					
16	2045	AA111329	10^7 899-6+RT	6.5	5.5	4		23.4					
	2046	AA111329	10^7 899-6+RT	9	7.5	5.5		20.7					
	2047	AA111329	10^7 899-6+RT	7	6.5	4		22.0					
	2048	AA111330	10^7 899-6+RT	8	5.5	4.5		20.2					
	2049	AA111330	10^7 899-6+RT	14.5	10	9		20.5					
	2050	AA111330	10^7 899-6+RT	6	8	4		21.2					
	2051	AA111330	10^7 899-6+RT	11	9	6.5		20					
			10^7 899-6+RT										
		328											
	2068	AA111~~335~~	R 3616	8.5	7	5	111 328	18.1					
	2069	AA111335	R 3616	8	4	7	104549	23.2					
	2070	AA111335	R 3616	10.5	7	6	"	23.3					
	2071	AA111336	R 3616	8	7.5	5.5	111325	23.0					
	2072	AA111336	I R3616 + RT	7	6	4		23.8	111	326			
	2073	AA111336	I R3616 + RT	9.5	8	6.5		20.6	111	327			
	2074	AA111336	I 3616 + RT	9	6	7		17	[illegible]	166			
	2075	AA111337	I 899-6	9	10	5		21.7	105	283			
	2076	AA111337	I 899-6	8.5	8.5	5.5		23.8	111	332			
	2077	AA111337	I 899-6 induction			inj	3/13		111	337			
	2078	AA111337	I 899-6 induction			"			"				

In vivo TNF induction

PURPOSE - To see if TNFα ~~induction~~ production is induced in vivo with 20Gy delivered 24° after injection of U87 hind limb tumors with R899-6 (10^7 PFU)

METHODS -

1. Implant subcut tumors in hind limb of nude mice
2. Grow to ~100 mm^3 in size
3. Inject with 2×10^7 PFU R899-6 (10 ul of stock)
4. 24 hours after injection randomize to +/- 20Gy, and sacrifice at the following intervals:

Time	⊖RT	⊕RT
2° + 24°	4 mice	4 mice
→ 6° + 24°	"	"
→ 12° + 24°	"	"
→ 24° + 24°	"	"
→ 48° + 24°	"	"
→ 1 wk + 24°	"	"
→ 2 wk + 24°	"	"
3 wk + 24°	"	"

Need 64 mice

Infect	W	9AM
RT	Th	9AM
Sac @		~~F~~ 3PM
		9AM
	F	9AM

INDUCTION- in vivo							Note: Old standard curve data used				
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor	Tot Vol	TNF/ml	TNF/tumor	Mean	TNF/mg-tum	Mean
1702	Control	None		63	0.06	0.66	18	12	817	199	703
1698	Control	None		2520	2.5	3.5	477	1668		667	
1724	Control	None		67	0.07	0.67	23	15		219	
2080	Control 1	None		938	0.9	1.5	259	388		432	
2081	Control 2	None		1124	1	2	1000	2000		2000	
2042	3616			528	0.6	1.2	252	303	261	505	408
2034	3616+20+25			644	0.7	1.3	168	218		312	
2077	899-6			2090	2.9	3.9	56	219	664	75	161
2078	899-6			6186	4.5	5.5	202	1109		246	
2145	2 hrs + RT				0.51	1.11	214	238	127	466	573
2146	2 hrs + RT				0.39	0.99	81	80		206	
2147	2 hrs + RT				0.06	0.66	95	63		1047	
2161	6 hrs + RT				0.7	1.3	158	205	156	293	271
2162	6 hrs + RT				0.66	1.26	144	182		276	
2163	6 hrs + RT				0.68	1.28	124	158		233	
2164	6 hrs + RT				0.28	0.88	90	79		283	
2177	6 hrs no RT				1.03	2.03	246	499	261	484	663
2178	6 hrs no RT				0.5	1.1	138	151		303	
2179	6 hrs no RT				0.23	0.83	191	159		690	
2180	6 hrs no RT				0.2	0.8	293	235		1174	
2185	12 hrs + RT				0.5	1.1	196	216	158	431	307
2186	12 hrs + RT				0.54	1.14	83	95		176	
2187	12 hrs + RT				0.47	1.07	81	87		185	
2188	12 hrs + RT				0.54	1.14	206	235		436	
2141	12 hrs no RT				0.19	0.79	122	96	111	506	466
2142	12 hrs no RT				0.2	0.8	173	138		691	
2143	12 hrs no RT				0.09	0.69	51	35		389	
2144	12 hrs no RT				0.64	1.24	142	176		276	
2169	24 hrs + RT				0.08	0.68	91	62	157	774	839
2170	24 hrs + RT				0.79	1.39	167	232		294	
2171	24 hrs + RT				0.1	0.7	142	99		993	
2172	24 hrs + RT				0.18	0.78	298	233		1293	
2165	24 hrs no RT				0.19	0.79	264	209	166	1098	917
2166	24 hrs no RT				0.11	0.71	106	75		684	
2167	24 hrs no RT				0.34	0.94	182	171		504	
2168	24 hrs no RT				0.15	0.75	277	207		1383	
2181	48 hrs + RT				0.34	0.94	204	191	252	563	464
2182	48 hrs + RT				0.73	1.33	355	472		647	
2183	48 hrs + RT				0.46	1.06	211	224		487	
2184	48 hrs + RT				0.74	1.34	89	119		161	
2173	48 hrs no RT				0.34	0.94	174	164	138	482	480
2174	48 hrs no RT				0.24	0.84	197	166		691	
2175	48 hrs no RT				0.64	1.24	126	156		244	
2176	48 hrs no RT				0.13	0.73	90	66		505	
2153	1 wk + RT				0.53	1.13	56	64	85	120	133
2154	1 wk + RT				0.57	1.17	72	84		148	
2155	1 wk + RT				0.98	1.58	84	133		136	
2156	1 wk + RT				0.47	1.07	57	61		129	
2157	1 wk no RT				0.06	0.66	34	22	66	371	291
2158	1 wk no RT				0.21	0.81	133	108		514	
2159	1 wk no RT				0.54	1.14	93	106		196	
2160	1 wk no RT				0.32	0.92	29	27		83	
2149	2 wk + RT				0.2	0.8	53	43	72	214	327
2150	2 wk + RT				0.5	1.1	72	80		159	
2151	2 wk + RT				0.17	0.77	191	147		867	
2152	2 wk + RT				0.26	0.86	21	18		69	
2137	2 wk no RT				3.36	4.36	59	256	142	76	91
2138	2 wk no RT				0.73	1.33	50	66		91	
2139	2 wk no RT				1.12	2.12	46	98		87	
2140	2 wk no RT				1.35	2.35	63	149		110	

IN VIVO TNF INDUCTION

	1	2	3	4	5	6	7	8	9
A	1000		2161	6°	2185	12°	2169	24°	2181
B	500		2162	+	2186	+	2170	+	2182
C	250		2163	RT	2187	RT	2171	~~RT~~	2183
D	125		2164		2188		2172		2184
E	62		2177		2141		2165		2173
F	31		2178	6°	2142	12°	2166	24°	2174
G	16		2179	no RT	2143	no	2167	~~no~~	2175
H	0		2180		2144	RT	2168	RT	2176

	10	11	12	13	14	15	16	17	18
A	48°	2153	1wk	2149	*2wk	1702	*control	2081	control 2
B	+RT	2154	+	$\boxed{2150}$	*+	1698	control	2145	2° RT
C		2155	RT	2151	*RT	1724	control	2146	2° RT
D		2156		2152	*	2042	*8616	2147	2° RT
E	48°	2157	*	2137	2wk	2034	*8616+RT	2081	
F	no RT	2158	* 1wk	2138	no	2077	899-6	2145	
G		2159	no	2139	RT	2078	89, 6	2146	
H		2160	RT	2140		2080	control 1	*2147	

	19	
A	10^7 6°	+RT
B	10^7 6°	no RT
C	10^8 6°	+RT
D	10^8 6°	no RT
E	10^7 24°	+RT
F	10^7 24°	no RT
G	10^8 24°	+RT
H	10^8 24°	no RT

not all supernatant assayed due was spilled

* for these sets, there was insufficient supernatant to fill the second well

649 x (C491.84)

READ MODE

OPTICAL DENSITY

1	2	3	4	5	6	7	8	9	10	11	12
		6° + RT		12° + RT		24° + RT		48° + RT		1 wk + RT	
		6° ⊖ RT		12° ⊖ RT		24° no RT		48° ⊖ RT		1 wk ⊖ RT	

READ MODE

[blot] = invalid data

OPTICAL DENSITY

13	14	15	16	17	18	19	20
2 wk + RT						10^7	
						10^8	
2 wk no RT						10^7	
						10^8	

INDUCTION- in vivo							
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor Wt	Vol Buffer	[TNF]pg/ml
1702	Control	None		63	0.06	.6	0.37
~~1698~~	Control	None		67	~~0.07~~ 2.0	1.0	1.03
1724	Control	None		2520	~~2.5~~ 0.07	.6	0.42
2042	3616			528	0.6	.6	0.33
2034	3616+20+25			644	0.7	.6	0.41
2077	899-6			2090	2.9	1.0	~~0.41~~ 0.89
2078	899-6			6186	4.5	1.0	0.44
2080	Control 1			938	0.9	.6	0.52
2081	Control 2			1124	1	1.0	1.31
2145	2 hrs + RT				0.51	.6	0.57
2146	2 hrs + RT				0.39	.6	0.49
2147	2 hrs + RT				0.06	.6	0.40

8

52
.6
5.2
2
39.2

40ml 150mM NaCl
400 ul TRIS 1M pH 7.5
400 ul EDTA 0.5M pH 7.5

protease inhibitors

/ DTT 1mM 40ul
/ PMSF 100mM 20ul
leupeptin 5ug 4ul
aprotinin 10ug 8ul

26 — = 34 ml
.52

76
2
8|152 wells

19 rows
8
152

2

wt. supernatant

INDUCTION- in vivo							
Mouse	**Treatment**	**Rx Date**	**Sac Date**	**Size**	**Tumor Wt**	**Vol Buffer**	**[TNF]pg/ml**
2161	6 hrs + RT				0.7	.6	.58
2162	6 hrs + RT				0.66	.6	0.47
2163	6 hrs + RT				0.68	.6	.53
2164	6 hrs + RT				0.28	.6	0.50
2177	6 hrs no RT				1.03	1.0	0.93
2178	6 hrs no RT				0.5	.6	1.45
2179	6 hrs no RT				0.23	.6	~~0.44~~ 0.52
2180	6 hrs no RT				0.2	.6	0.40
2185	12 hrs + RT				0.5	.6	.55 mg
2186	12 hrs + RT				0.54	.6	.500
2187	12 hrs + RT				0.47	.6	0.55
2188	12 hrs + RT				0.54	.6	0.52
2141	12 hrs no RT				0.19	.6	0.49
2142	12 hrs no RT				0.2	.6	.46
2143	12 hrs no RT				0.09	.6	0.42
2144	12 hrs no RT				0.64	.6	0.53
2169	24 hrs + RT				0.08	.6	0.57
2170	24 hrs + RT				0.79	.6	0.26
2171	24 hrs + RT				0.1	.6	0.38
2172	24 hrs + RT				0.18	.6	0.42
2165	24 hrs no RT				0.19	.6	0.56
2166	24 hrs no RT				0.11	.6	0.39
2167	24 hrs no RT				0.34	.6	0.51
2168	24 hrs no RT				0.15	.6	0.58
2181	48 hrs + RT				0.34	.6	0.56
2182	48 hrs + RT				0.73	.6	0.66
2183	48 hrs + RT				0.46	.6	0.61
2184	48 hrs + RT				0.74	.6	0.73
2173	48 hrs no RT				0.34	.6	0.52
2174	48 hrs no RT				0.24	.6	0.56
2175	48 hrs no RT				0.64	.6	0.73
2176	48 hrs no RT				0.13	.6	0.42
2153	1 wk + RT				0.53	.6	0.59
2154	1 wk + RT				0.57	.6	0.43
2155	1 wk + RT				0.98	.6	0.73
2156	1 wk + RT				0.47	.6	0.40
2157	1 wk no RT				0.06	.6	0.34
2158	1 wk no RT				0.21	.6	0.48
2159	1 wk no RT				0.54	.6	0.46
2160	1 wk no RT				0.32	.6	0.42
2149	2 wk + RT				0.2	.6	0.38
2150	2 wk + RT				0.5	.6	0.32 ← spilled some supernat
2151	2 wk + RT				0.17	.6	0.28
2152	2 wk + RT				0.26	.6	~~0.38~~
2137	2 wk no RT				3.36	1.0	1.25
2138	2 wk no RT				0.73	.6	0.58
2139	2 wk no RT				1.12	1.0	0.89
2140	2 wk no RT				1.35	1.0	0.77

600 ul RIPA buffer added to tumors < 1 gm
1000 ul " " " " " ≥ 1 gm

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 18784

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Greg Sibley, MD DATE:

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: [signature]

FAS ACCOUNT: 2-73731-5100 P.I.: Hallahan

VENDOR: FCRI PROTOCOL: 58671

REQUESTED DELIVERY DATE: PHONE: 2-0294

SPECIES: Mouse QUANTITY: 80

STRAIN: Athymic Nude SEX: M (F) EITHER

WEIGHT/AGE: 5-6 weeks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

HOUSE AT: ___ CARLSON ___ WYLER X CLSC ___ FMI ___ OTHER

1863	AA108971	10^7 3616+RT	10.5	8	3							
1864	AA108971	10^7 3616+RT	6	8	3.5							
1865	AA108971	10^7 3616+RT	3	3	2							
1866	AA108971	10^7 3616+RT	3	3.5	1.5							
1883	AA108966	10^7 3616+RT	4.5	4	1							
1884	AA108966	10^7 3616+RT	7.5	6	5							
1886	AA108966	10^7 3616+RT	6	4	3							
2074	AA108966	10^7 3616+RT	6.5	5	7							
2032	AA111326	10^7 3616+RT	10	6	5.5							
2033	AA111326	10^7 3616+RT	12	9	5.5							
2035	AA111326	10^7 3616+RT	8.5	7.5	6							
2072	AA111326	10^7 3616+RT	13	9.5	8							
2036	AA111327	10^7 3616+RT	12	9	6							
2037	AA111327	10^7 3616+RT	8.5	8	6							
2039	AA111327	10^7 3616+RT	10	9	5							
2073	AA111327	10^7 3616+RT	12.5	11.5	9							
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	6.5	7	4							
1872	AA108969	10^7 899-6+RT	4.5	6.5	2.5							
1873	AA108969	10^7 899-6+RT	8	8	3.5							
1874	AA108969	10^7 899-6+RT	7	6	3							
1879	AA108967	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	3.5	3	1							
1881	AA108967	10^7 899-6+RT	6	4.5	2							
1882	AA108967	10^7 899-6+RT	3	2.5	1							
2044	AA111329	10^7 899-6+RT	9.5	8	5							
2045	AA111329	10^7 899-6+RT	5.5	5	3							
2046	AA111329	10^7 899-6+RT	9.5	8	5							
2047	AA111329	10^7 899-6+RT	6	6	3.5							
2048	AA111330	10^7 899-6+RT	7.5	6	4.5							
2049	AA111330	10^7 899-6+RT	15	10	8							
2050	AA111330	10^7 899-6+RT	7	6.5	4							
2051	AA111330	10^7 899-6+RT	11	13	5							
		10^7 899-6+RT										

The University of Chicago Departmental Purchase Order

Z 890001
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
Sarstedt
St James Church Rd
Newton, NC 28658
City State Zip Code
Payment Terms ______ Delivery charge? ☐ Yes ☒ No
Telephone No. 800-257-5101 FAX No. ______

Ship To
THE UNIVERSITY OF CHICAGO Dept. Code: ______
Radiation Oncology MC-0085
Department
~~G-03~~ Greg Sibley 6-03
Attn: Room
CHGO IL 60637
City State Zip Code

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? ☐ No ☒ Yes
Mary
Order placed with (name) Date
Greg Sibley 800-257-5101
Order placed by (name) Phone #

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1000	65816 Dome stopper	19.22	
1	1000	55.518 13 ml centrifuge tubes	57.92	
			77.14	
			ORDER TOTAL	

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1720	AA105283	10^7 899-6	25	17.5	14		26.5					
1710	AA105283	10^7 899-6	0	0	0		22.7					
2075	AA105283	10^7 899-6	10	9	3		20.7					
1713	AA105283	10^7 899-6	0	0	0		21.2					
2052	AA111331	10^7 899-6	14.5	12	8		20.3					
2053	AA111331	10^7 899-6	12	15.5	8		22.7					
2054	AA111331	10^7 899-6	19	15	6		19.7					
2055	AA111331	10^7 899-6	11	7	3.5		21.9					
2056	AA111332	10^7 899-6	12	15	7		24.5					
2057	AA111332	10^7 899-6	6	6	3		21.4					
2058	AA111332	10^7 899-6	12.5	11	6		23.9					
2076	AA111332	10^7 899-6	9.5	11	7		24.6					
2060	AA111333	10^7 899-6	~~19~~	~~17.5~~	~~4.5~~		~~25.3~~	12.5	12.5	8		23.7
2061	AA111333	10^7 899-6	11	10	5		22.6					
2062	AA111333	10^7 899-6	8	6	4		16.9					
2063	AA111333	10^7 899-6	9	8	6.5		21.0					
		10^7 899-6										
1697	AA104549	10^7 3616	19	17.5	9.5		25.3					
1708	AA104549	10^7 3616	0	0	0		21.9					
1709	AA104549	10^7 3616	0	0	0		27.3					
1723	AA104549	10^7 3616	0	0	0		26.3					
1877	AA108968	10^7 3616										
1878	AA108968	10^7 3616										
2041	AA111328	10^7 3616	6	5	3		22.3					
2043	AA111328	10^7 3616	13.5	12	7		25					
2064	AA111334	10^7 3616	8	6.5	4		23.1					
2065	AA111334	10^7 3616	16	16	9		23.3					
2066	AA111334	10^7 3616	12	12	6		23.2					
2067	AA111334	10^7 3616	8.5	6	3.5		14.5					
2068		10^7 3616	7	6	5		15.8					
2069		10^7 3616	10	10	7.5		23.2					
2070		10^7 3616	11	8	5		23.4					
2071		10^7 3616	11	10	5		24.0					
		10^7 3616										
1859	AA108972	RT Alone	9.5	10	3.5		23.9					
~~1860~~	AA108972	RT Alone	3/27/195									
1861	AA108972	RT Alone	12	13.5	7		21.5					
1862	AA108972	RT Alone	8	7	3		25.3					
1892	AA108964	RT Alone	7.5	8	5		22.8					
		RT Alone										
			~~12~~	~~13.5~~	~~7~~		~~21.5~~					

1863	AA108971	10^7 3616+RT	10	7	2.5		22.9					
1864	AA108971	10^7 3616+RT	7.5	5	3		24.1					
1865	AA108971	10^7 3616+RT	3.5	3	1		24.9					
1866	AA108971	10^7 3616+RT	3	3	1		24.9					
1883	AA108966	10^7 3616+RT	0	0	0		22					
1884	AA108966	10^7 3616+RT	5.5	5.5	3		20.6					
1886	AA108966	10^7 3616+RT	11.5	10	7		14.8					
2074	AA108966	10^7 3616+RT	4	4	1		22					
2032	AA111326	10^7 3616+RT	11.5	7.5	5		20.2					
2033	AA111326	10^7 3616+RT	11.5	9	6		21.6					
2035	AA111326	10^7 3616+RT	5.5	5.5	3		27.7					
2072	AA111326	10^7 3616+RT	13	10	6		18.8					
2036	AA111327	10^7 3616+RT	12	9	4		20.4					
2037	AA111327	10^7 3616+RT	6	8	4		23.6					
2039	AA111327	10^7 3616+RT	11	8	7.5		19.3					
2073	AA111327	10^7 3616+RT	8.5	10	5		17.9					
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	7	7	3.5		23.3					
1872	AA108969	10^7 899-6+RT	4.5	6	3		22.4					
1873	AA108969	10^7 899-6+RT	7.5	8	4		22.4					
1874	AA108969	10^7 899-6+RT	6.5	5	2.5		24.8					
1879	AA108967	10^7 899-6+RT	0	0	0		26.0					
1880	AA108967	10^7 899-6+RT	0	0	0		20.7					
1881	AA108967	10^7 899-6+RT	0	0	0		24.4					
1882	AA108967	10^7 899-6+RT	4	3	1		22.1					
2044	AA111329	10^7 899-6+RT	9	7.5	5.5		20.9					
2045	AA111329	10^7 899-6+RT	7	5	3		24.5					
2046	AA111329	10^7 899-6+RT	8.5	7	5		22.7					
2047	AA111329	10^7 899-6+RT	5	5	5		21.2					
2048	AA111330	10^7 899-6+RT	5.5	8	3.5		19.2					
2049	AA111330	10^7 899-6+RT	15	10.5	5		21.8					
2050	AA111330	10^7 899-6+RT	6	6	4		21.4					
2051	AA111330	10^7 899-6+RT	17.5	11	4.5		20.5					
		10^7 899-6+RT										

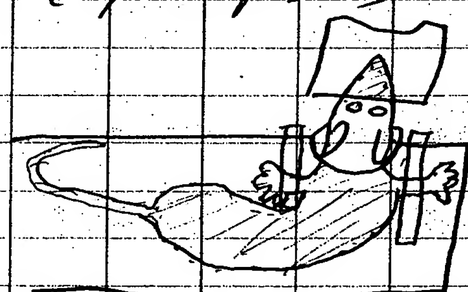
TOLERANCE OF NUDE MICE TO WHOLE BRAIN RT

PURPOSE - In preparation for stereotactic brain protocol, need to determine maximum tolerated whole brain dose

PROCEDURE -

① Anesthetize mice c̄ Ketamine / Xylazine 0.4 ml

② Tape front legs of mice with head protruding through lead shield & piece of lead over nose (eyes exposed)



③ Irradiate c̄ Maxitron: Plexiglass-to-coll dist = 8 cm, 30 mA 150 KVp (see TLD measurements next page)

TLD @ surface of skull = 450-500 cGy
TLD under head = 325 cGy
} 500 cGy intended

④ Pilot experiment = 3 mice

mouse #1 = 1000 x 5 = 5000 cGy
#2 = 500 x 6 = 3000 cGy
#3 = [illegible]0 x 8 = 4000 cGy

RESULTS -

	RT dates start	end	Status
mouse #1			died
mouse #2			
mouse #3			

TLD exposure Measurement on Mice for

Dr. Sidhu & Hyland Channel .035" TLDs

100 KVp

30 mA Beam larger than for bottom of collimator

1 mm Al filter

Calibration TLDs

500 cGy at above beam quality

11.38 μC

11.29 11.59 ± .60

12.33

11.38 $\left(\frac{TL}{D}\right)_{100KVp} = \frac{11.59\ \mu C}{5.00\ Gy} = 2.32\ \frac{\mu C}{Gy}$

TLDs on Mice

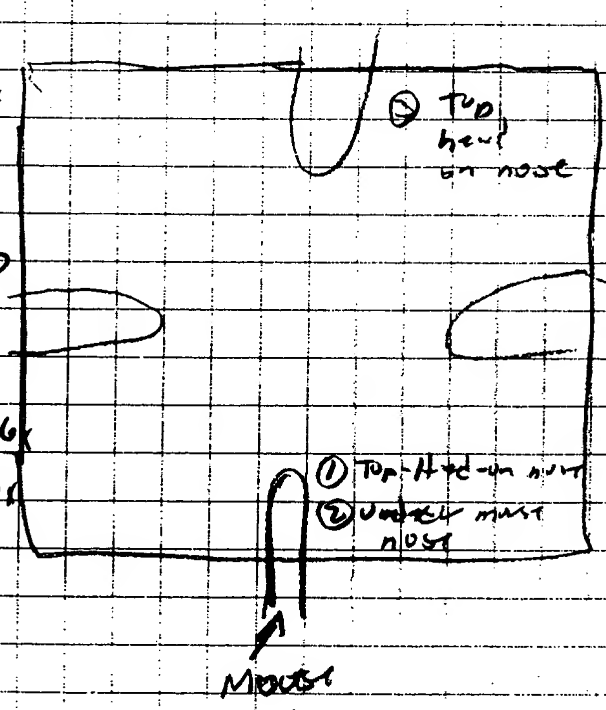
① 11.54 μC 497 cGy

10.82 466 cGy <482 cGy>

② 6.81 μC 294 cGy

8.27 356

<325 cGy>



③ 11.18 482 cGy

9.60 414 cGy

<448>

The University of Chicago Departmental Purchase Order

Purchase Order Number
Z890593
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
Fisher Scientific
1600 W Glenlake Ave
Itasca, IL 60143
City / State / Zip Code
Payment Terms ______ Delivery charge? ☐ Yes ☐ No
Telephone No. 2-3328 FAX No. ______

Ship To
THE UNIVERSITY OF CHICAGO Dept. Code: ______
U of C Rad Onc
Department: Greg Sibley Room: G-08
Attn: 5830 S. Ellis MC 0085
City: Chgo State: IL Zip Code: 60637

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? ☐ No ☒ Yes
Order placed with (name): Cindy
Order placed by (name): Greg Sibley Phone #: 2-0294

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
4	ea	Hamilton Syringes 10 ul 14-824-3	23.04	92.16
2	Box	15 ml polypropylene tubes 14-959-70C	$ 83.40	166.80
				~~258.96~~
				258.96
			ORDER TOTAL	

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

INDUCTION- in vivo					
Mouse	Treatment	Date	Size	Sac Date	Tumor wt
1702	Control		63		
1698	Control		67.375		
1724	Control		2520		
2040	3616		died		
2042	3616				0.6
2034	3616+20+25			..	0.7
~~2038~~	~~3616+20+25~~				
ℓ 2077	899-6			22·19·10	2.9 gm
RR 2078	899-6			27·23.5·15.5	4.5
~~2079~~	~~899-6~~		died		
2080 control 1		15·12.5·10	..		0.9 gm
2081 2		14.5·15.5·10	..		1.0 gm

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
~~1720~~	AA105283	10^7 899-6	20.5	28	16.5		29.3					
1710	AA105283	10^7 899-6	0	0	0		23.6					
2075	AA105283	10^7 899-6	12	12	7		23.4					
1713	AA105283	10^7 899-6	0	0	0		21.6					
2052	AA111331	10^7 899-6	16.5	14.5	12		21.9					
2053	AA111331	10^7 899-6	21	15	11.5		24.1					
2054	AA111331	10^7 899-6	16	12	7.5		22.0					
~~2055~~	AA111331	10^7 899-6	23.5	21	10.5		22.75	sacked				
2056	AA111332	10^7 899-6	20	18	11		25.9					
2057	AA111332	10^7 899-6	9.5	8	6		21.8					
2058	AA111332	10^7 899-6	19	20	8.5		25.9					
2076	AA111332	10^7 899-6	15	16.5	9		23.5					
2060	AA111333	10^7 899-6	19.5	18	13		25.8					
2061	AA111333	10^7 899-6	20	16	11.5		26.2					
2062	AA111333	10^7 899-6	13.5	8	8.5		17.2					
2063	AA111333	10^7 899-6	17.5	13	10.5		23.5					
		10^7 899-6										
1697	AA104549	10^7 3616	18.5	18.5	13		25.2					
1708	AA104549	10^7 3616	0	0	0		21.4					
1709	AA104549	10^7 3616	0	0	6		25.7					
1723	AA104549	10^7 3616	0	0	0		26.0					
2069	AA108968	10^7 3616	12	15	8		24.8					
2070	AA108968	10^7 3616	13	11	6		26.2					
2041	AA111328	10^7 3616	8	8	5.5		23.6					
2043	AA111328	10^7 3616	22	17	11.5		26.5					
2068	AA111328	10^7 3616	5	4.5	3		14.9					
2071	AA111328	10^7 3616	15	12.5	8		26.3					
2064	AA111334	10^7 3616	8.5	8	5		24.1					
~~2065~~	AA111334	10^7 3616	23	24	14		25.8					
2066	AA111334	10^7 3616	16	15.5	6.5		25.2					
~~2067~~	AA111334	10^7 3616	8	6	4.5		12.4					
90		10^7 3616										
1859	AA108972	RT Alone	9	9	4		25.5					
1861	AA108972	RT Alone	12	14.5	8		20.5					
1862	AA108972	RT Alone	6.5	8	5		24.9					
1892	AA108964	RT Alone	7	6.5	3.5		22.7					
		RT Alone										

1863	AA108971	10^7 3616+RT	8.5	7	3		23.4					
1864	AA108971	10^7 3616+RT	6.5	4	4		24.4					
1865	AA108971	10^7 3616+RT	2	2	1		24.1					
1866	AA108971	10^7 3616+RT	3.5	3	1		25.0					
1883	AA108966	10^7 3616+RT	0	0	0		22.2					
1884	AA108966	10^7 3616+RT	4.5	4	2		22.7					
1886	AA108966	10^7 3616+RT	10	10	4		14.6					
2074	AA108966	10^7 3616+RT	0	0	0		22.3					
2032	AA111326	10^7 3616+RT	7	5.5	5.5		19.5					
2033	AA111326	10^7 3616+RT	11.5	8	6		21.2					
2035	AA111326	10^7 3616+RT	10.5	8.5	7		18.3					
2072	AA111326	10^7 3616+RT	4	5	2.5		22.1					
2036	AA111327	10^7 3616+RT	11	8.5	5		21.0					
2037	AA111327	10^7 3616+RT	6.5	5	3.5		24.5					
2039	AA111327	10^7 3616+RT	12.5	11	6		18.2					
2073	AA111327	10^7 3616+RT	7.5	7.5	7.5		18.9					
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	6	6	3		24.5					
1872	AA108969	10^7 899-6+RT	6	4.5	3.5		23.4					
1873	AA108969	10^7 899-6+RT	7	8.5	5		24.1					
1874	AA108969	10^7 899-6+RT	5.5	3.5	2		25.8					
1879	AA108967	10^7 899-6+RT	0	0	0		26.4					
1880	AA108967	10^7 899-6+RT	0	0	0		22.1					
1881	AA108967	10^7 899-6+RT	0	0	0		23.4					
1882	AA108967	10^7 899-6+RT	0	0	0		24.7					
2044	AA111329	10^7 899-6+RT	6.5	7.5	4		23.2					
2045	AA111329	10^7 899-6+RT	3	3.5	1.5		25.2					
2046	AA111329	10^7 899-6+RT	6.5	6	4		24.2					
2047	AA111329	10^7 899-6+RT	0	0	0		22.4					
2048	AA111330	10^7 899-6+RT	4.5	3.5	2.5		20.0					
2049	AA111330	10^7 899-6+RT	12.5	11	7		23.4					
2050	AA111330	10^7 899-6+RT	6	5	4		23.5					
2051	AA111330	10^7 899-6+RT	9	6.5	4		23.2					
		10^7 899-6+RT										

1863	AA108971	10^7 3616+RT	8.5	7	4							
1864	AA108971	10^7 3616+RT	8.5	6	5							
1865	AA108971	10^7 3616+RT	3	3	1							
1866	AA108971	10^7 3616+RT	~~3.5~~	3	+	0	0	0				
1883	AA108966	10^7 3616+RT	5	4	1							
1884	AA108966	10^7 3616+RT	3.5	3	2.5							
1886	AA108966	10^7 3616+RT	0	0	0							
2074	AA108966	10^7 3616+RT	9.5	10.5	4.5							
2032	AA111326	10^7 3616+RT	6.5	5	4.5							
2033	AA111326	10^7 3616+RT	11	8	6							
2035	AA111326	10^7 3616+RT	11	8.5	6.5							
2072	AA111326	10^7 3616+RT	6	4	1							
2036	AA111327	10^7 3616+RT	10	9.5	4.5							
2037	AA111327	10^7 3616+RT	4.5	6	2.5							
2039	AA111327	10^7 3616+RT	10	12	7.5							
2073	AA111327	10^7 3616+RT	7	6.5	3							
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	6.5	6	3							
1872	AA108969	10^7 899-6+RT	~~6~~ 4	~~4~~ 6	~~4~~ 3.5							
1873	AA108969	10^7 899-6+RT	7	7.5	4.5							
1874	AA108969	10^7 899-6+RT	3	3.5	1.5							
1879	AA108967	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	4	5.5	1.5							
1881	AA108967	10^7 899-6+RT	4	3	1							
1882	AA108967	10^7 899-6+RT	0	0	0							
2044	AA111329	10^7 899-6+RT	~~7.5~~	6	~~3.5~~	6	6.5	4				
2045	AA111329	10^7 899-6+RT	3.5	3	1							
2046	AA111329	10^7 899-6+RT	7.5	6	3.5							
2047	AA111329	10^7 899-6+RT	3	3	1							
2048	AA111330	10^7 899-6+RT	4	4	3							
2049	AA111330	10^7 899-6+RT	13	11	7							
2050	AA111330	10^7 899-6+RT	3	5	2.5	edema						
2051	AA111330	10^7 899-6+RT	6.5	9	4							
		10^7 899-6+RT										
2082	AA111338	S brain RT	1000 x 5 died									
2083	AA111339	S "	500 x 8									
2084	AA111339	S "	500 x 6									
2085	AA111339	S stereotactic										
2086	AA111339	S "										
2087	AA111340	S "										
2088	AA111340	S "										
2089	AA111340	S										
2090	AA111340	S										

Greg's Mouse Log

	Mouse #	Cage #	Group										
	1710	AA105283	10^7 899-6	0	0	0							
	2075	AA105283	10^7 899-6	16	14	8.5							
	1713	AA105283	10^7 899-6	0	0	0							
5G	~~2052~~	AA111331	10^7 899-6	23	17.5	13							
	~~2053~~	AA111331	10^7 899-6	18.5	12	13	Sacked						
	2054	AA111331	10^7 899-6	28	14.5	13	16.5	12	8.5				
	~~2056~~	AA111332	10^7 899-6	21.5	15.5	12							
33←	2057	AA111332	10^7 899-6	9.5	21	6.5							
	~~2058~~	AA111332	10^7 899-6	19.5	21.5	10.5							
33←	2076	AA111332	10^7 899-6	17	18.5	9.5							
	~~2061~~	AA111333	10^7 899-6	23	18.5	12.5							
	2062	AA111333	10^7 899-6	15.5	11	10							
	2063	AA111333	10^7 899-6	20.5	16	11.5							
			10^7 899-6										
14	~~1697~~	AA104549	10^7 3616	18.5	19	12							
	1708	AA104549	10^7 3616	0	0	0							
	1709	AA104549	10^7 3616	0	0	0							
	1723	AA104549	10^7 3616	0	0	0							
4←	2069	AA108968	10^7 3616	13.5	17	~~10~~	7.5						
14	2070	AA108968	10^7 3616	13	16	17.5	remeasure						
	2041	AA111328	10^7 3616	10.5	9.5	6.5							
	2068	AA111328	10^7 3616	4.5	4	2.5							
	2071	AA111328	10^7 3616	18	16.5	10							
	2064	AA111334	10^7 3616	9	10	7							
	2066	AA111334	10^7 3616	20	17	9							
			10^7 3616										
	1859	AA108972	RT Alone	10	10	4							
	1861	AA108972	RT Alone	12	13	10.5							
	1862	AA108972	RT Alone	6.5	7	4							
	1892	AA108964	RT Alone	7.5	7.5	3.5							
			RT Alone										

~~9 10 5.5 14.4~~

1863	AA108971	10^7 3616+RT	9	6.5	4		24.2					
1864	AA108971	10^7 3616+RT	9	8.5	7		24.0					
1865	AA108971	10^7 3616+RT	2	2	1		24.1					
1866	AA108971	10^7 3616+RT	0	0	0		25.8					
1883	AA108966	10^7 3616+RT	0	0	0		21.8					
1884	AA108966	10^7 3616+RT	2	2	2		20.9					
1886	AA108966	10^7 3616+RT	0	0	0		21.8					
2074	AA108966	10^7 3616+RT	9	10	5.5		14.4					
2032	AA111326	10^7 3616+RT	9.5	7	5.5		20.9					
2033	AA111326	10^7 3616+RT	6	5	4		18.2					
2035	AA111326	10^7 3616+RT	13.5	12	6		18.5					
2072	AA111326	10^7 3616+RT	0	0	0		22.7					
2036	AA111327	10^7 3616+RT	10.5	9	4		20.7					
2037	AA111327	10^7 3616+RT	5	4	2.5		23.7					
2039	AA111327	10^7 3616+RT	8	7	4.5		18.6					
2073	AA111327	10^7 3616+RT	10	9	4.5		18.2					
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	6	5.5	2.5		19.4					
1872	AA108969	10^7 899-6+RT	4.5	6	4		23.5					
1873	AA108969	10^7 899-6+RT	7	7.5	2.5		24.0					
1874	AA108969	10^7 899-6+RT	3	2	1		26.1					
1879	AA108967	10^7 899-6+RT	0	0	0		27.2					
1880	AA108967	10^7 899-6+RT	0	0	0		20.7					
1881	AA108967	10^7 899-6+RT	0	0	0		25.2					
1882	AA108967	10^7 899-6+RT	0	0	0		23.7					
2044	AA111329	10^7 899-6+RT	4.5	5	3.5		23.2					
2045	AA111329	10^7 899-6+RT	0	0	0		25.8					
2046	AA111329	10^7 899-6+RT	6.5	5.5	4		23.7					
2047	AA111329	10^7 899-6+RT	0	0	0		22.5					
2048	AA111330	10^7 899-6+RT	3.5	3	2.5		18.0					
2049	AA111330	10^7 899-6+RT	6.5	9	4.5		22.0					
2050	AA111330	10^7 899-6+RT	3	4	2.5		22.4					
2051	AA111330	10^7 899-6+RT	12.5	11	8		23.0					
		10^7 899-6+RT										
2082	AA111338	S										
2083	AA111339	S										
2084	AA111339	S										
2085	AA111339	S										
2086	AA111339	S										
2087	AA111340	S										
2088	AA111340	S										
2089	AA111340	S										
2090	AA111340	S										

Greg's Mouse Log

Mouse #	Cage #	Group										
1710	AA105283	10^7 899-6	0	0	0		23.7					
2075	AA105283	10^7 899-6	17	19.5	9		24.1					
1713	AA105283	10^7 899-6	0	0	0		21.5					
2052	AA111331	10^7 899-6										
2053	AA111331	10^7 899-6										
2054	AA111331	10^7 899-6	11	15	8.5		19.4					
2056	AA111332	10^7 899-6										
2057	AA111332	10^7 899-6	11.5	9	6.5		21.0					
2058	AA111332	10^7 899-6										
2076	AA111332	10^7 899-6	20.5	17.5	13.5		28.4	sacked				
2061	AA111333	10^7 899-6										
2062	AA111333	10^7 899-6	17	11	8.5		16.2	sacked		face	tumor	
2063	AA111333	10^7 899-6	23	17.5		14.5	24.1	sacked				
		10^7 899-6										
1697	AA104549	10^7 3616										
face ~~1708~~	AA104549	10^7 3616	0	0	0		20.2	sacked				
1709	AA104549	10^7 3616	0	0	0		25.8					
1723	AA104549	10^7 3616	0	0	0		25.4					
2069	AA108968	10^7 3616	21	16.5	11		24.4					
2070	AA108968	10^7 3616	20	16	10		27.2					
2041	AA111328	10^7 3616	10	11	7.5		22.4	sacked				
2068	AA111328	10^7 3616						died				
2071	AA111328	10^7 3616	23	20.5	13		27.5					
2064	AA111334	10^7 3616	13	12.5	7		24.5					
~~2066~~	AA111334	10^7 3616	23	19	12							
		10^7 3616										
1859	AA108972	RT Alone	10.5	10	6		25.5					
1861	AA108972	RT Alone	13.5	13	8		21.2					
1862	AA108972	RT Alone	7	7	4		25.6					
1892	AA108964	RT Alone	7	6.5	3		22.8					
		RT Alone										

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

We placed our mice on Bactrim in Cummings Rm #1053 (on) due to the appearance of declining health and a number of deaths after tumor implantation. I apologize for not making you aware of this. Please continue Bactrim therapy for 1 week (until).

The mice placed on antibiotics include all mice with HALLAHAN/SIBLEY on the card (20 cages).

Cage #'s:

Sorry for the confusion.

Sincerely,

Gregory S. Sibley, M.D.

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0							
2075	AA105283	10^7 899-6	21.5	19.5	9							
1713	AA105283	10^7 899-6	0	0	0							
2054	AA111331	10^7 899-6	11	14	4.5							
2057	AA111332	10^7 899-6	13	10	7							
1709	AA104549	10^7 3616	0	0	0							
1723	AA104549	10^7 3616	0	0	0							
~~2069~~	AA108968	10^7 3616	23	20	13.5							
~~2070~~	AA108968	10^7 3616	23	19	11							
2064	AA111334	10^7 3616	15	14	7.5							
2041	"	10^7 3616	12	10	9							
1859	AA108972	RT Alone	11	10	6							
1861	AA108972	RT Alone	13.5	12.5	8							
1862	AA108972	RT Alone	6	6	3.5							
1892	AA108964	RT Alone	6	6	3.5							
1863	AA108971	10^7 3616+RT	8	6	4							
1864	AA108971	10^7 3616+RT	12	10	9							
1865	AA108971	10^7 3616+RT	0	0	0							
1866	AA108971	10^7 3616+RT	0	0	0							
1883	AA108966	10^7 3616+RT	0	0	0							
1884	AA108966	10^7 3616+RT	3	3.5	2							
1886	AA108966	10^7 3616+RT	0	0	0							
2074	AA108966	10^7 3616+RT	9	10	5							
2032	AA111326	10^7 3616+RT	6	5	5							
2033	AA111326	10^7 3616+RT	9	7	3.5							
2035	AA111326	10^7 3616+RT	~~8~~ 9	7	6							
2072	AA111326	10^7 3616+RT	0	0	0							
2036	AA111327	10^7 3616+RT	10	9	4							
2037	AA111327	10^7 3616+RT	5	3.5	2.5							
2039	AA111327	10^7 3616+RT	7	8	3.5							
2073	AA111327	10^7 3616+RT	9.5	9	5							
1871	AA108969	10^7 899-6+RT	6.5	6.5	4							
1872	AA108969	10^7 899-6+RT	4	5	3.5							
1873	AA108969	10^7 899-6+RT	7	7	4.5							
1874	AA108969	10^7 899-6+RT	0	0	0							
1879	AA108967	10^7 899-6+RT	0		0							
1880	AA108967	10^7 899-6+RT	0		0							
1881	AA108967	10^7 899-6+RT	[illegible]		0							
1882	AA108967	10^7 899-6+RT	0		0							
2044	AA111329	10^7 899-6+RT	4.5	4.[illegible]	3.5							
2045	AA111329	10^7 899-6+RT	0	0	0							
2046	AA111329	10^7 899-6+RT	6.5	6	3							
2047	AA111329	10^7 899-6+RT	0	0	0							
2048	AA111330	10^7 899-6+RT	3	3.5	2							
2049	AA111330	10^7 899-6+RT	14	12	10							
2050	AA111330	10^7 899-6+RT	3	2.5	1							
2051	AA111330	10^7 899-6+RT	9	6.5	4.5							

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0		23.0					
2075	AA105283	10^7 899-6	21	18	6.5		21.9					
1713	AA105283	10^7 899-6	0	0	0		21.6					
2054	AA111331	10^7 899-6	11	15	4		19.2					
2057	AA111332	10^7 899-6	14.5	12	8.5		21.1					
1709	AA104549	10^7 3616	0	0	0		23.9					
1723	AA104549	10^7 3616	0	0	0		24.4					
2069	AA108968	10^7 3616										
2070	AA108968	10^7 3616										
2064	AA111334	10^7 3616	15.5	14	8		23.4					
2041		3616	13.5	12	9		21.9					
1859	AA108972	RT Alone	11.5	10	5		26.1					
1861	AA108972	RT Alone	6.5	6	4.5		24.5					
1862	AA108972	RT Alone	14	14	7.5		22.5					
1892	AA108964	RT Alone	6.5	6.5	3.5		22.8					
1863	AA108971	10^7 3616+RT	8	5	3		24.5					
1864	AA108971	10^7 3616+RT	12	11	8.5		25.6					
1865	AA108971	10^7 3616+RT	0	0	0		26.4					
1866	AA108971	10^7 3616+RT	0	0	0		25.8					
1883	AA108966	10^7 3616+RT	0	0	0		22.0					
1884	AA108966	10^7 3616+RT	3	3	2		22.3					
1886	AA108966	10^7 3616+RT	10	9	4.5		16.4					
2074	AA108966	10^7 3616+RT	0	0	0		21.5					
2032	AA111326	10^7 3616+RT	6	5	5		19.9					
2033	AA111326	10^7 3616+RT	6	5.5	3.5		22.4					
2035	AA111326	10^7 3616+RT	8	6	4		19.3					
2072	AA111326	10^7 3616+RT	0	0	0		22.7					
2036	AA111327	10^7 3616+RT	10	9	4		22.5					
2037	AA111327	10^7 3616+RT	4	5	2.5		24.0					
2039	AA111327	10^7 3616+RT	8	8	4		19.4					
2073	AA111327	10^7 3616+RT	8	9	5		19.4					
1871	AA108969	10^7 899-6+RT	5	3	2		25.4					
1872	AA108969	10^7 899-6+RT	6.5	5.5	3		22.6					
1873	AA108969	10^7 899-6+RT	7	7.5	5		23.3					
1874	AA108969	10^7 899-6+RT	0	0	0		26.1					
1879	AA108967	10^7 899-6+RT	0	0	0		28.7					
1880	AA108967	10^7 899-6+RT	0	0	0		21.9					
1881	AA108967	10^7 899-6+RT	0	0	0		25.6					
1882	AA108967	10^7 899-6+RT	0	0	0		23.6					
2044	AA111329	10^7 899-6+RT	4	4	3.5		22.8					
2045	AA111329	10^7 899-6+RT	0	0	0		25.6					
2046	AA111329	10^7 899-6+RT	[illegible].5	6	3		23.7					
2047	AA111329	10^7 899-6+RT	0	0	0		21.7					
2048	AA111330	10^7 899-6+RT	4	3	2		18.3					
2049	AA111330	10^7 899-6+RT	14	13	6		22.6					
2050	AA111330	10^7 899-6+RT	3	3	2		22.7					
2051	AA111330	10^7 899-6+RT	8	10	3.5		21.7					

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	ϕ									
2075	AA105283	10^7 899-6	20	15.5	6							
1713	AA105283	10^7 899-6	ϕ									
2054	AA111331	10^7 899-6	14	9.5	4							
2057	AA111332	10^7 899-6	15	11.5	8							
1709	AA104549	10^7 3616	ϕ									
1723	AA104549	10^7 3616	ϕ									
~~2069~~	~~AA108968~~	~~10^7 3616~~										
~~2070~~	~~AA108968~~	~~10^7 3616~~										
2064	AA111334	10^7 3616	15.5	13.5	10.5							
2041	AA111334	10^7 3616	14	12	9							
1859 ϕ	AA108972	RT Alone	11	9.5	7							
1861 R	AA108972	RT Alone face	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	14.5	13	12.5				
1862 LR	AA108972	RT Alone	5.5	5	3.5							
1892 (L)	AA108964	RT Alone necrotic tail	6.5	6	3.5							
1863 ϕ	AA108971	10^7 3616+RT	6	5	4							
1864 L	AA108971	10^7 3616+RT	11	10	9.5							
1865 R	AA108971	10^7 3616+RT	ϕ									
1866 LR	AA108971	10^7 3616+RT	ϕ									
1883 ϕ	AA108966	10^7 3616+RT	ϕ									
1884 L	AA108966	10^7 3616+RT	2	2	2							
1886 LR	AA108966	10^7 3616+RT	ϕ									
2074 R	AA108966	10^7 3616+RT	8.5	10	5							
2032 ϕ	AA111326	10^7 3616+RT	4.5	5	4.5							
2033 L	AA111326	10^7 3616+RT	5.5	5	3							
2035 LR	AA111326	10^7 3616+RT	8	7	5							
2072 R	AA111326	10^7 3616+RT	ϕ									
2036 ϕ	AA111327	10^7 3616+RT	8.5	8	5							
2037 L	AA111327	10^7 3616+RT	4.5	4	2.5							
2039 LR	AA111327	10^7 3616+RT	8	7	6							
2073 R	AA111327	10^7 3616+RT	6.5	7	3							
1871 ϕ	AA108969	10^7 899-6+RT	ϕ									
1872 L	AA108969	10^7 899-6+RT	2.5	2.5	2							
1873 R	AA108969	10^7 899-6+RT	7	7.5	6							
1874 LR	AA108969	10^7 899-6+RT	ϕ									
~~1879~~	~~AA108967~~	~~10^7 899-6+RT~~										
1880 L	AA108967	10^7 899-6+RT	ϕ									
1881 R	AA108967	10^7 899-6+RT	ϕ									
1882 LR	AA108967	10^7 899-6+RT	ϕ									
2044 ϕ	AA111329	10^7 899-6+RT	4.5	4	2.5							
2045 L	AA111329	10^7 899-6+RT	ϕ									
2046 R	AA111329	10^7 899-6+RT	6.5	6	3							
2047 LR	AA111329	10^7 899-6+RT	ϕ									
2048 ϕ	AA111330	10^7 899-6+RT	3	3	2							
2049 L	AA111330	10^7 899-6+RT	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	13	12	10				
2050 R	AA111330	10^7 899-6+RT	~~[illegible]~~	ϕ								
2051 LR	AA111330	10^7 899-6+RT	10	9	6							

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0		23.3					
2075	AA105283	10^7 899-6	21	17	8		22.4					
1713	AA105283	10^7 899-6	0	0	0		21.4					
2054	AA111331	10^7 899-6	13.5	6.5	5		20.5					
2057	AA111332	10^7 899-6	18.5	16.5	14.5		23.3					
1709	AA104549	10^7 3616	0	0	0		24.0					
1723	AA104549	10^7 3616	0	0	0		23.5					
2041	AA111334	10^7 3616	18	16	14		24.8					
2064	AA111334	10^7 3616	18.5	16.5	12		23.5					
1859	AA108972	RT Alone	12	9.5	8.5		26.1					
~~1861~~	AA108972	RT Alone sac										
1862	AA108972	RT Alone	5.5	4.5	3.5		25.1					
1892	AA108964	RT Alone	5.5	5.5	3.5		24.1					
1863	AA108971	10^7 3616+RT	5	4.5	3.5		25.0					
1864	AA108971	10^7 3616+RT	11	14	11		25.1					
1865	AA108971	10^7 3616+RT	0	0	0		25.9					
1866	AA108971	10^7 3616+RT	0	0	0		25.3					
1883	AA108966	10^7 3616+RT	0	0	0		22.2					
1884	AA108966	10^7 3616+RT	2	2	1		24.4					
1886	AA108966	10^7 3616+RT	0	0	0		21.3					
2074	AA108966	10^7 3616+RT	9	9	6		16.3					
2032	AA111326	10^7 3616+RT	4.5	5.5	3.5		22.0					
2033	AA111326	10^7 3616+RT	5.5	4.5	3.5		24.8					
2035	AA111326	10^7 3616+RT	8	6.5	5		21.7					
2072	AA111326	10^7 3616+RT	0	0	0		24.2					
2036	AA111327	10^7 3616+RT	8	7	4		23.9					
2037	AA111327	10^7 3616+RT	4	4.5	1.5		24.6					
2039	AA111327	10^7 3616+RT	9	9	8		20.3					
2073	AA111327	10^7 3616+RT	7	7	4		20.2					
1871	AA108969	10^7 899-6+RT	5	5	3		23.3					
~~1872~~	AA108969	10^7 899-6+RT	ascites									
1873	AA108969	10^7 899-6+RT	6.5	8	5.5		23.8					
1874	AA108969	10^7 899-6+RT	0	0	0		26.1					
1880	AA108967	10^7 899-6+RT	0	0	0		22.4					
1881	AA108967	10^7 899-6+RT	0	0	0		25.4					
1882	AA108967	10^7 899-6+RT	0	0	0		24.4					
2044	AA111329	10^7 899-6+RT	3.5	3.5	3		23.3					
2045	AA111329	10^7 899-6+RT	0	0	0		25.9					
2046	AA111329	10^7 899-6+RT	5.5	6	2.5		24.8					
~~2047~~	AA111329	10^7 899-6+RT	0	0	0		22.1					
2048	AA111330	10^7 899-6+RT	2.5	2.0	1		17.4					
2049	AA111330	10^7 899-6+RT	13.5	11	9		24.7					
2050	AA111330	10^7 899-6+RT	4	3	2		23.2					
2051	AA111330	10^7 899-6+RT	8.5	8.0	6.5		22.9					

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0							
2075	AA105283	10^7 899-6	16	12	3							
1713	AA105283	10^7 899-6	0	0	0							
2054	AA111331	10^7 899-6	11	5	3.5							
~~2057~~	AA111332	10^7 899-6	20	17.5	15							
1709	AA104549	10^7 3616	0	0	0							
~~1723~~	AA104549	10^7 3616	0	0	0							
~~2041~~	AA111334	10^7 3616	19	17	15							
~~2064~~	AA111334	10^7 3616	17.5	17	13.5							
1859	AA108972	RT Alone	12	11	8							
~~1861~~	AA108972	RT Alone										
1862	AA108972	RT Alone	5	4	3.5							
1892	AA108964	RT Alone	6	5	4							
1863	AA108971	10^7 3616+RT	5	5	3.5							
1864	AA108971	10^7 3616+RT	13	9.5	5.5							
1865	AA108971	10^7 3616+RT	0	0	0							
1866	AA108971	10^7 3616+RT	0	0	0							
1883	AA108966	10^7 3616+RT	0	0	0							
1884	AA108966	10^7 3616+RT	2	2.5	1							
1886	AA108966	10^7 3616+RT	0	0	0							
2074	AA108966	10^7 3616+RT	9.5	8.5	5							
2032	AA111326	10^7 3616+RT	4.5	4	3.5							
2033	AA111326	10^7 3616+RT	5.5	5	3							
2035	AA111326	10^7 3616+RT	8	6.5	4.5							
2072	AA111326	10^7 3616+RT	0	0	0							
2036	AA111327	10^7 3616+RT	8	7	4							
2037	AA111327	10^7 3616+RT	4	4	2							
2039	AA111327	10^7 3616+RT	9.5	8	6							
2073	AA111327	10^7 3616+RT	7	7	3							
1871	AA108969	10^7 899-6+RT	5.5	5	3							
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT	7.5	6	5							
1874	AA108969	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0							
1881	AA108967	10^7 899-6+RT	0	0	0							
1882	AA108967	10^7 899-6+RT	0	0	0							
2044	AA111329	10^7 899-6+RT	4	3.5	3							
2045	AA111329	10^7 899-6+RT	0	0	0							
2046	AA111329	10^7 899-6+RT	5	5	2.5							
2047	AA111329	10^7 899-6+RT										
2048	AA111330	10^7 899-6+RT	2	3	1.5							
2049	AA111330	10^7 899-6+RT	16	13	10							
2050	AA111330	10^7 899-6+RT	2	3	2							
2051	AA111330	10^7 899-6+RT	9	8	5.5							

58
17
126
18

306
13
918

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0		24.0					
2075	AA105283	10^7 899-6	0	0	0		21.8					
1713	AA105283	10^7 899-6	3	2	1		21.8					
2054	AA111331	10^7 899-6	0	0	0		21.8					
2057	AA111332	10^7 899-6										
1709	AA104549	10^7 3616										
1723	AA104549	10^7 3616	0	0	0		23.7					
2041	AA111334	10^7 3616										
2064	AA111334	10^7 3616										
1859	AA108972	RT Alone	13	11.5	8		23.9					
1861	AA108972	RT Alone										
1862	AA108972	RT Alone	4.5	5	4		25.8					
1892	AA108964	RT Alone	5	5.5	4		24.3					
1863	AA108971	10^7 3616+RT	4.5	4	3		24.6					
1864	AA108971	10^7 3616+RT	13	10.5	7.5		26.3					
1865	AA108971	10^7 3616+RT	2.5	2.5	1		26.5					
1866	AA108971	10^7 3616+RT	3	3.5	1		25.8					
1883	AA108966	10^7 3616+RT	0	0	0		22.6					
1884	AA108966	10^7 3616+RT	1.5	1.5	1.5		24.3					
1886	AA108966	10^7 3616+RT	0	0	0		21.4					
2074	AA108966	10^7 3616+RT	9.5	8	6		17.3					
2032	AA111326	10^7 3616+RT	7	5	4		21.4					
2033	AA111326	10^7 3616+RT	4.5	4	3		24.3					
2035	AA111326	10^7 3616+RT	8	7	5.5		21.4					
2072	AA111326	10^7 3616+RT	0	0	0		23.5					
2036	AA111327	10^7 3616+RT	7	7	3		22.9					
2037	AA111327	10^7 3616+RT	3.5	3	2		24.7					
2039	AA111327	10^7 3616+RT	9	8	6.5		18.3					
2073	AA111327	10^7 3616+RT	6	5.5	2		19.5					
1871	AA108969	10^7 899-6+RT	4	4	3		23.7					
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT	6.5	6.5	3		23.6					
1874	AA108969	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0		21.8					
1881	AA108967	10^7 899-6+RT	0	0	0		26.6					
1882	AA108967	10^7 899-6+RT	0	0	0		23.5					
2044	AA111329	10^7 899-6+RT	4	3.5	2.5		22.9					
2045	AA111329	10^7 899-6+RT	0	0	0		25.9					
2046	AA111329	10^7 899-6+RT	4.5	4	3		23.8					
2047	AA111329	10^7 899-6+RT										
2048	AA111330	10^7 899-6+RT	3.5	3	1.5		16.8					
2049	AA111330	10^7 899-6+RT	15.5	13.5	11		26.4					
2050	AA111330	10^7 899-6+RT	2.5	2	1		23.2					
2051	AA111330	10^7 899-6+RT	9	7.5	5		22.6					

Apoptosis Assay

Purpose - To determine whether apoptosis plays a role in U-87 cell killing with TNF, RT, R3616 virus or R899-6 virus or combinations of these

Methods - 8 T150 flasks of U87 cells were grown to ~80% confluence.

		7am			1pm		5pm	1pm
		-6°	-4°	-2°	0°	2°	4°	→ 24°
	Control (no R_x)							Harvest
	TNF (100 ng/20 ml)				TNF			Harvest
	RT (20Gy) + TNF				TNF		RT	Harvest
	RT (20Gy)						RT	Harvest
3.8 ul	R3616 (10^7 PFU) + RT	infect					RT	Harvest
	R3616 (10^7 PFU)	infect						Harvest
2.6 ul	R899-6 (10^7 PFU) + RT	infect					RT	Harvest
	R899-6 (10^7 PFU)	infect						

1. Virally infected ~~cells~~ flasks were ~~a~~ treated with 10^7 PFU of virus (MOI approx 0.5) in 6 ml 199V media. Other flasks ~~received~~ incubated c 6 ml of 199V s virus as control.

2. At 2 hours, an additional ~~24~~ 14 ml of ~~10%~~ FCS media was added to bring total volume to ~~30~~ 20 cc
(actually I added 10% FCS by mistake x 3 hrs, switched to ØFCS @ 12N, this required decanting media from infected flasks)

3. Add TNF to appropriate flasks = .01 mg/ml stock = .01 ug/ul
add 10 ul (100 ng) to 20 ml

APOPTOSIS ASSAY

Sample	Apoptosis (%)
Baseline	2.3
Positive Control	80.4
No Treatment (-TdT)	0.3
No Treatment	1.9
RT 20 Gy	19.3
TNF	2.6
TNF + RT	19
R3616 (MOI=0.5)	29.3
R3616 + RT	23.2
R899-6 (MOI=0.5)	26.8
R899-6 + RT	11.4

U87

Neg. control
No TdT

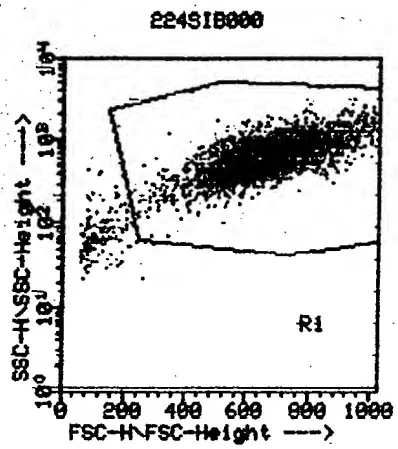
BECTON
DICKINSON

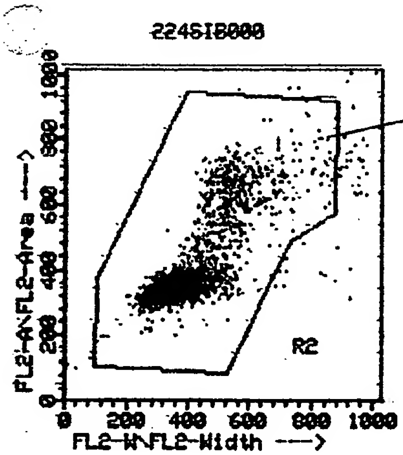
LYSYS II Ver 1.1

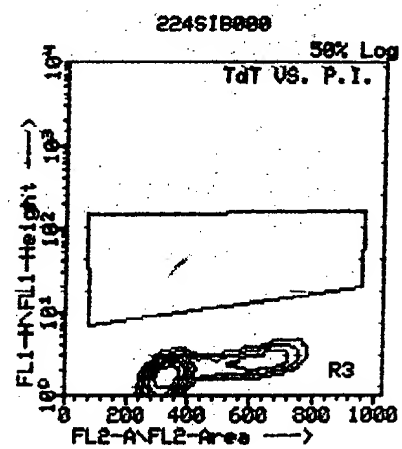
DATE:

TIME: 14:54:28

SELECTED PREFERENCES: Arithmetic/Linear





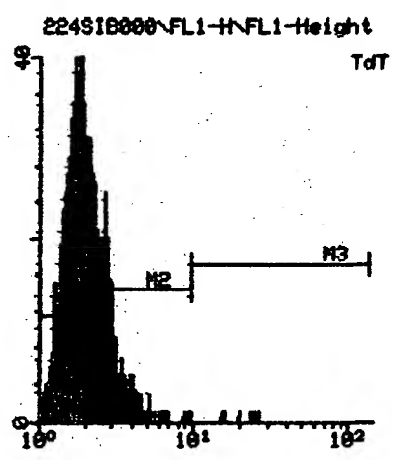


224SIB000

----- Region Stats -----

File: 224SIB000 Sample: GREG SIBLE'
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2368

Rgn	Events	% Gated	% Total
1 R1	2368	100.00	78.93
2 R2	2368	100.00	78.93
3 R3	8	0.34	0.27



224SIB000\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB000
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2368	100.00	43	1.91
1	1.00, 2.81	2108	89.02	43	1.84
2	2.81, 9.82	273	11.53	20	3.16
3	9.82, 136	7	0.30	1	19.81

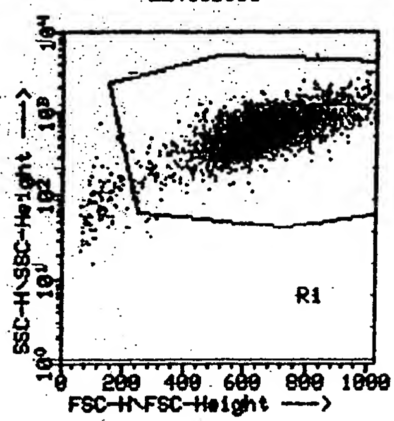
Control

BECTON DICKINSON LYSYS II Ver 1.1

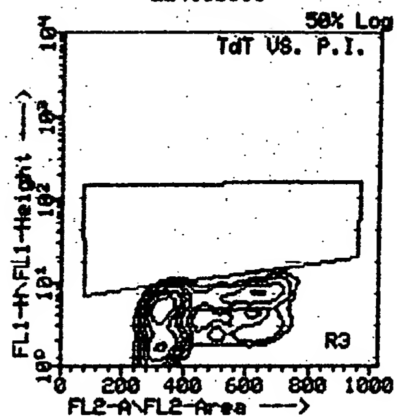
DATE: TIME: 14:56:24

SELECTED PREFERENCES: Arithmetic/Linear

224SIB001

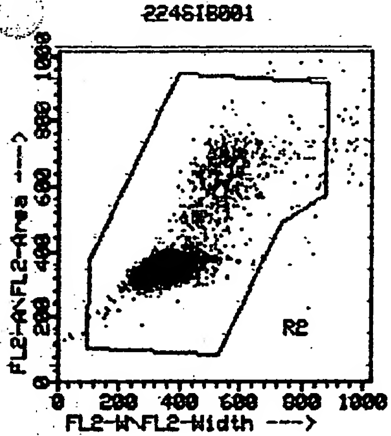


224SIB001



serum depleted

224SIB001



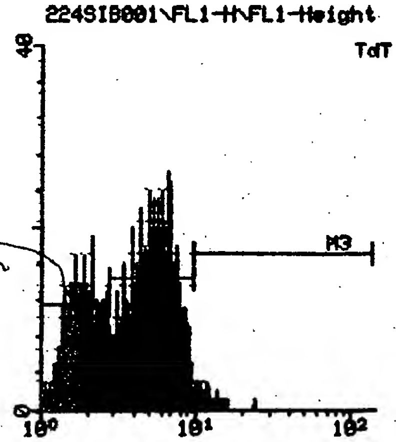
224SIB001

---- Region Stats ----

File: 224SIB001 Sample: GREG SIBLE
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2514

Rgn	Events	% Gated	% Total
1 R1	2514	100.00	83.80
2 R2	2514	100.00	83.80
3 R3	13	0.52	0.43

224SIB001\FL1-H\FL1-Height



Chromatin condensation

224SIB001\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB001
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2514	100.00	26	4.33
1	1.00, 2.81	819	32.58	19	1.91
2	2.81, 9.82	1657	65.91	26	5.33
3	9.82, 136	48	1.91	3	11.09

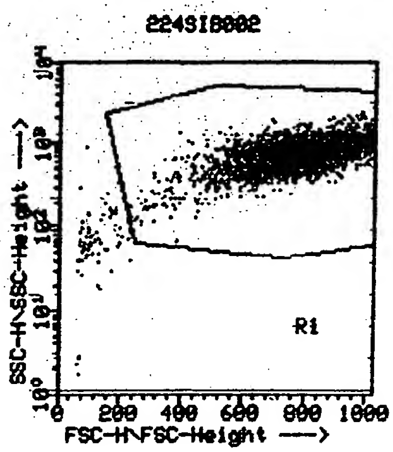
RT

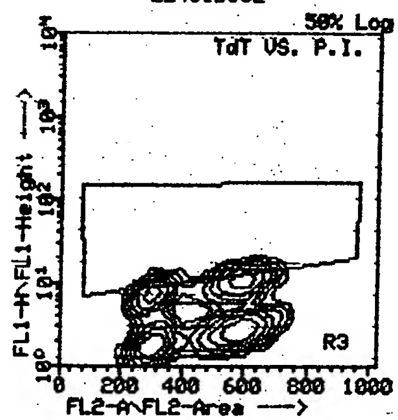
BECTON
DICKINSON

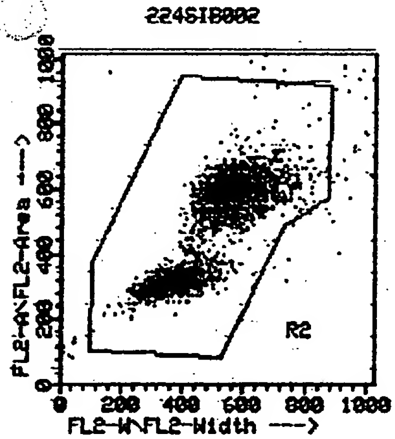
LYSYS II Ver 1.1

DATE: TIME: 14:58:36

SELECTED PREFERENCES: Arithmetic/Linear







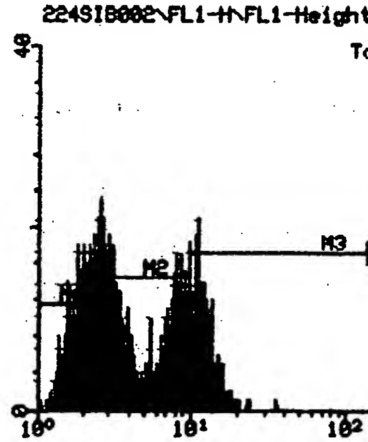
224SIB002

---- Region Stats ----

File: 224SIB002 Sample: GREG SIBLE'
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2343

Rgn	Events	% Gated	% Total
1 R1	2343	100.00	78.10
2 R2	2343	100.00	78.10
3 R3	209	8.92	6.97

224SIB002\FL1-H\FL1-Height



224SIB002\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB002
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2343	100.00	23	3.34
1	1.00, 2.81	937	39.99	23	2.15
2	2.81, 9.82	974	41.57	19	5.03
3	9.82, 136	451	19.25	21	11.06

TNF

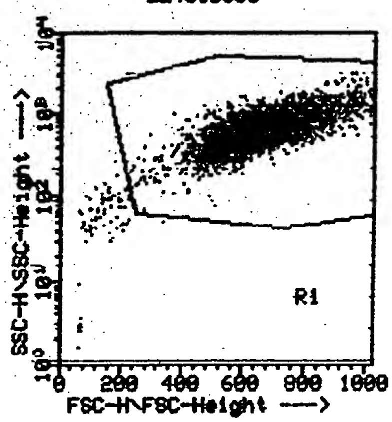
BECTON
DICKINSON

LYSYS II Ver 1.1

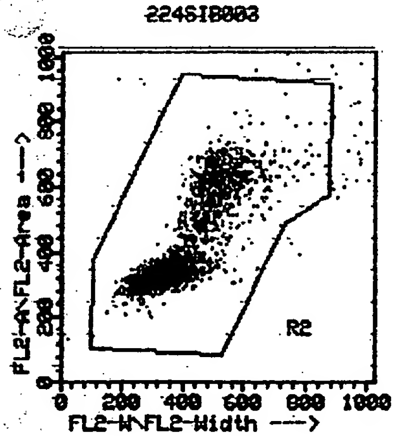
DATE: TIME: 15:00:12

SELECTED PREFERENCES: Arithmetic/Linear

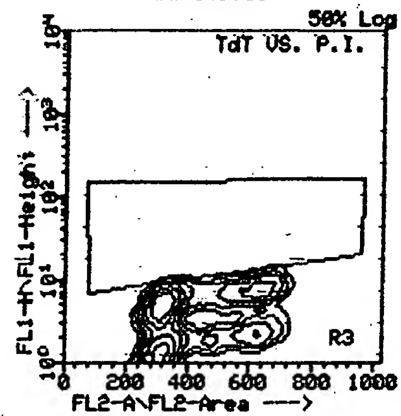
224SIB003



224SIB003



224SIB003



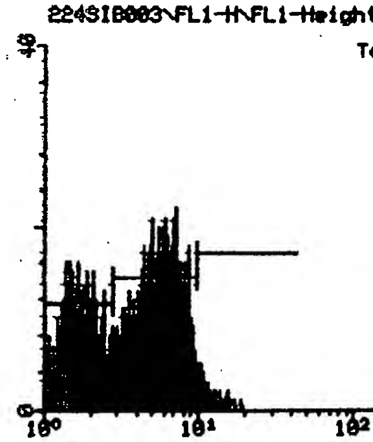
224SIB003

----- Region Stats -----

File: 224SIB003 Sample: GREG SIBLE'
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2533

Rgn	Events	% Gated	% Total
1 R1	2533	100.00	84.43
2 R2	2533	100.00	84.43
3 R3	17	0.67	0.57

224SIB003\FL1-H\FL1-Height



224SIB003\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 224SIB003
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2533	100.00	29	4.14
1	1.00, 2.81	953	37.62	29	1.65
2	2.81, 9.82	1522	60.09	22	5.57
3	9.82, 136	66	2.61	5	11.09

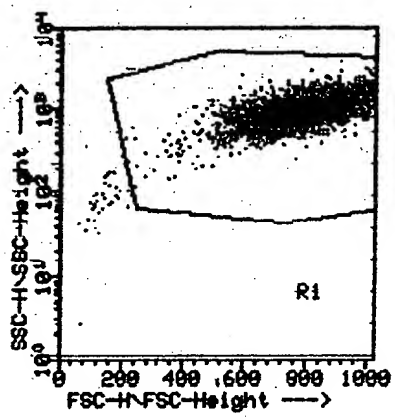
TNF+RT

BECTON DICKINSON LYSYS II Ver 1.1

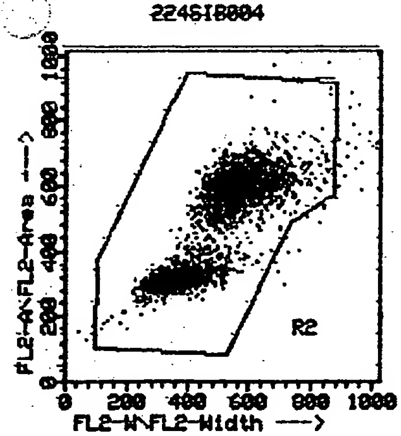
DATE: TIME: 15:01:05

SELECTED PREFERENCES: Arithmetic/Linear

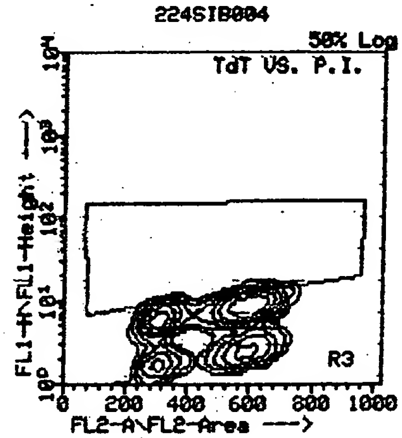
224SIB004



224SIB004



224SIB004



224SIB004

----- Region Stats -----

File: 224SIB004 Sample: GREG SIBLE

Date: Gate G2= R1ANDR2

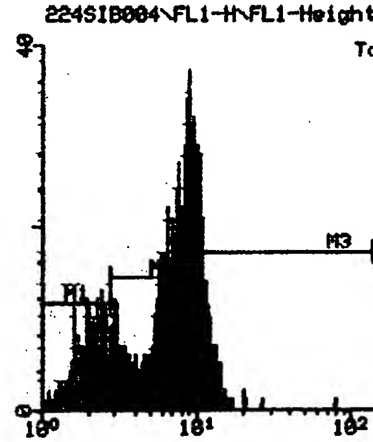
Selected Preference: Arithmetic/Lin

Parameters: FL2-A(LIN),FL1-H(LOG)

Total= 3000 Gated= 2530

Rgn	Events	% Gated	% Total
1 R1	2530	100.00	84.33
2 R2	2530	100.00	84.33
3 R3	61	2.41	2.03

224SIB004\FL1-H\FL1-Height



224SIB004\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB004

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2530	100.00	37	7.17
1	1.00, 2.01	511	20.20	13	2.07
2	2.01, 9.82	1571	62.09	37	7.30
3	9.82, 136	481	19.01	29	10.75

3616

BECTON
DICKINSON

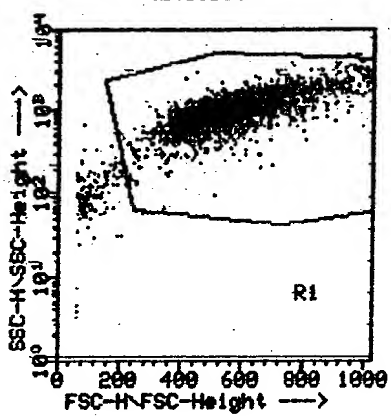
LYSYS II Ver 1.1

DATE:

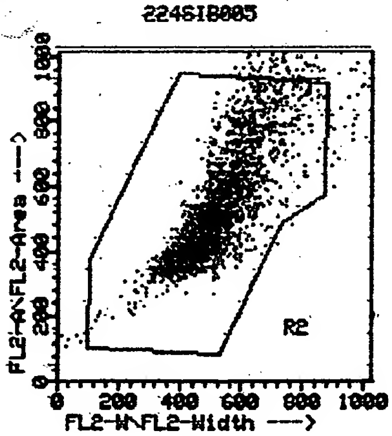
TIME: 15:01:58

SELECTED PREFERENCES: Arithmetic/Linear

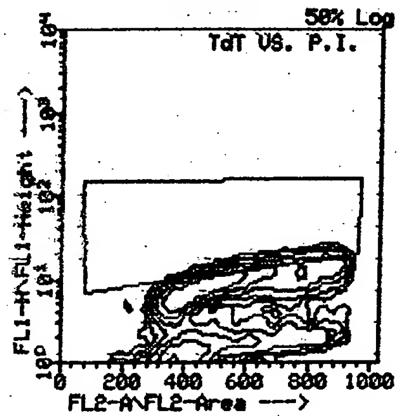
224SIB005



224SIB005



224SIB005



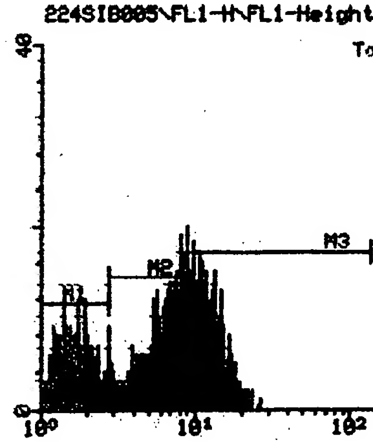
224SIB005

----- Region Stats -----

File: 224SIB005 Sample: GREG SIBLE
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2002

Rgn	Events	% Gated	% Total
1 R1	2002	100.00	66.73
2 R2	2002	100.00	66.73
3 R3	189	9.44	6.30

224SIB005\FL1-H\FL1-Height



224SIB005\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 224SIB005
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2002	100.00	20	7.17
1	1.00, 2.01	524	26.17	20	1.61
2	2.01, 9.02	909	45.40	20	6.98
3	9.02, 136	587	29.32	17	12.30

3616 + RT

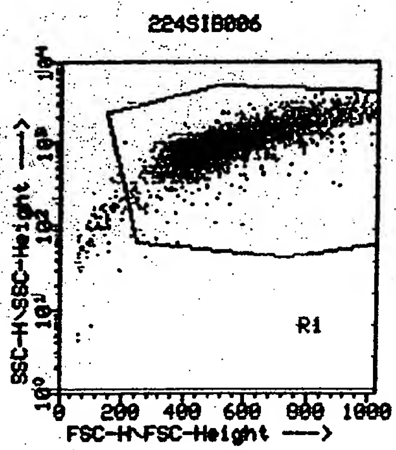
BECTON
DICKINSON

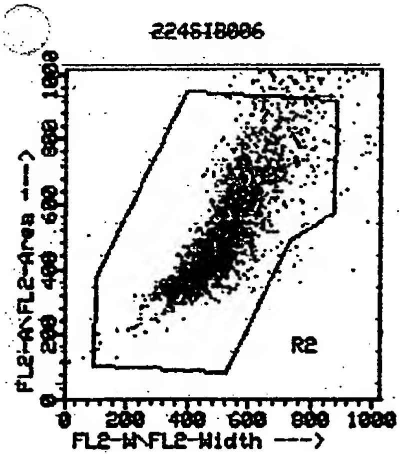
LYSYS II Ver 1.1

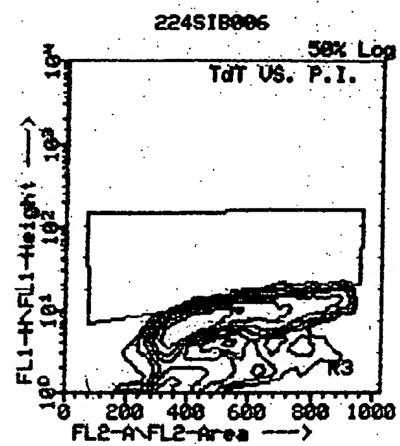
DATE:

TIME: 15:02:49

SELECTED PREFERENCES: Arithmetic/Linear





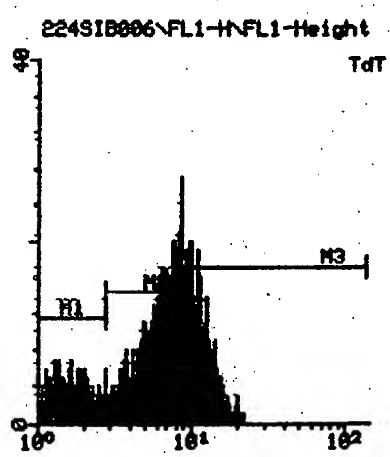


224SIB006

---- Region Stats ----

File: 224SIB006 Sample: GREG SIBLE'
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2017

Rgn	Events	% Gated	% Total
1 R1	2017	100.00	67.23
2 R2	2017	100.00	67.23
3 R3	74	3.67	2.47



224SIB006\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 224SIB006
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2017	100.00	27	7.04
1	1.00, 2.01	343	17.01	13	1.65
2	2.01, 9.82	1227	60.83	27	6.79
3	9.82, 136	467	23.15	20	11.65

899-6

BECTON DICKINSON

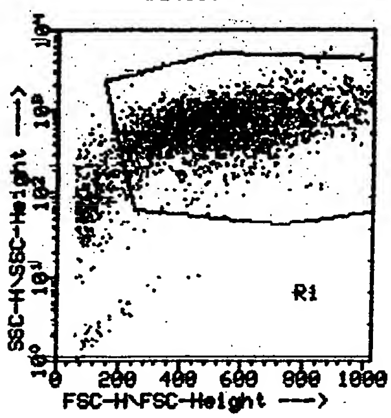
LYSYS II Ver 1.1

DATE:

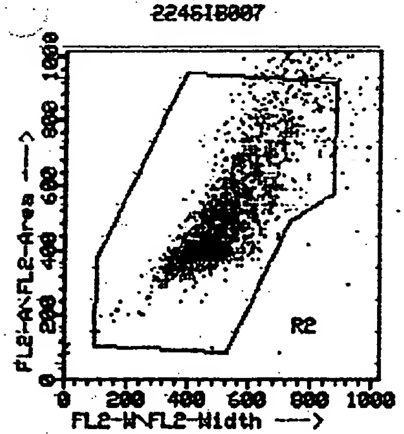
TIME: 15:03:40

SELECTED PREFERENCES: Arithmetic/Linear

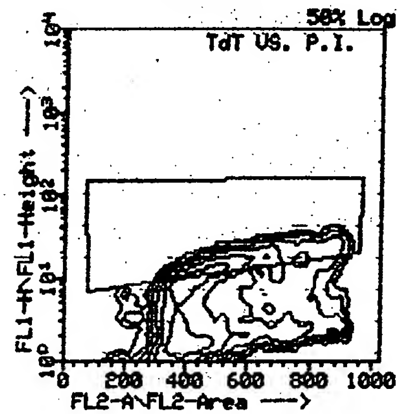
224SIB007



224SIB007



224SIB007



224SIB007

----- Region Stats -----

File: 224SIB007 Sample: GREG SIBLE

Date: Gate G2= R1ANDR2

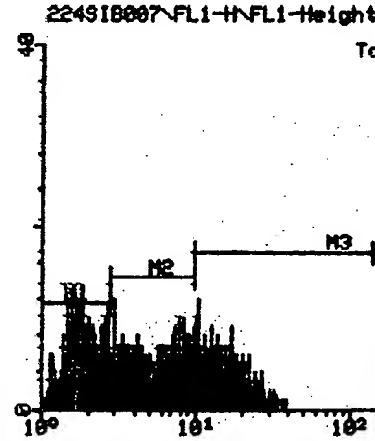
Selected Preference: Arithmetic/Lin

Parameters: FL2-A(LIN),FL1-H(LOG)

Total= 3000 Gated= 1710

Rgn	Events	% Gated	% Total
1 R1	1710	100.00	57.00
2 R2	1710	100.00	57.00
3 R3	366	21.40	12.20

224SIB007\FL1-H\FL1-Height



224SIB007\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB007

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1710	100.00	13	4.49
1	1.00, 2.81	599	35.03	13	1.84
2	2.81, 9.82	658	38.48	12	5.33
3	9.82, 136	459	26.84	12	14.20

899-6 + RT

BECTON DICKINSON

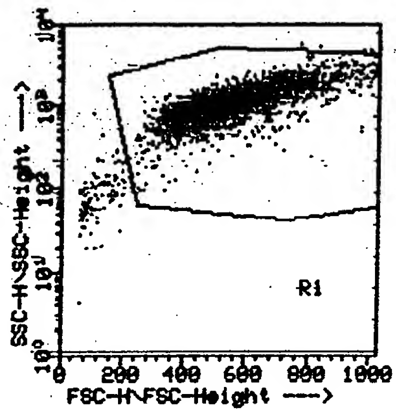
LYSYS II Ver 1.1

DATE:

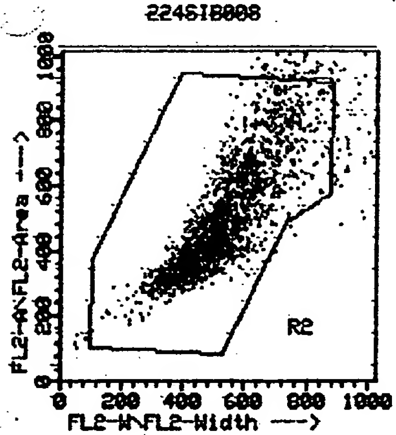
TIME: 15:04:34

SELECTED PREFERENCES: Arithmetic/Linear

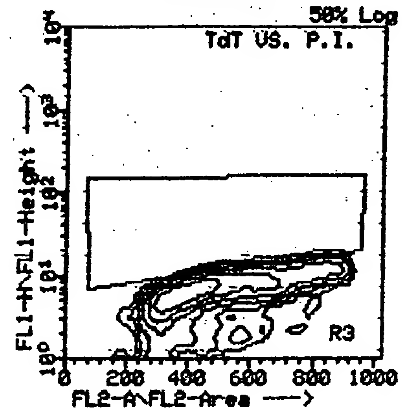
224SIB008



224SIB008



224SIB008



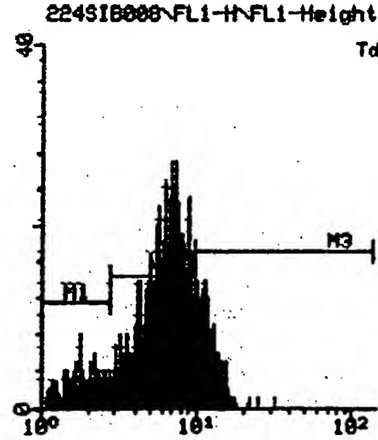
224SIB008

---- Region Stats ----

File: 224SIB008 Sample: GREG SIBLE
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2068

Rgn	Events	% Gated	% Total
1 R1	2068	100.00	68.93
2 R2	2068	100.00	68.93
3 R3	30	1.45	1.00

224SIB008\FL1-H\FL1-Height



224SIB008\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB008
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2068	100.00	27	6.73
1	1.00, 2.81	218	10.54	8	1.84
2	2.81, 9.82	1515	73.26	27	6.49
3	9.82, 136	349	16.88	14	11.44

Baseline for
Positive Control

BECTON DICKINSON

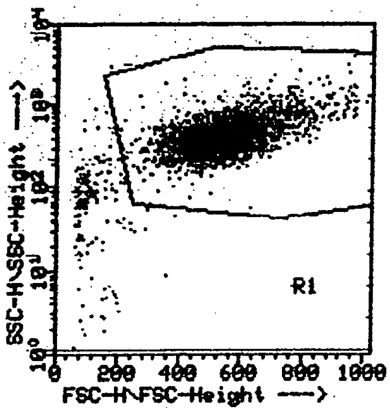
LYSYS II Ver 1.1

DATE:

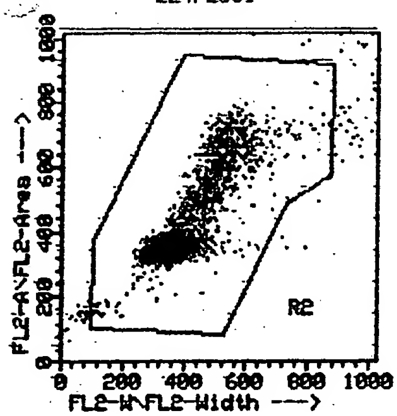
TIME: 15:11:28

SELECTED PREFERENCES: Arithmetic/Linear

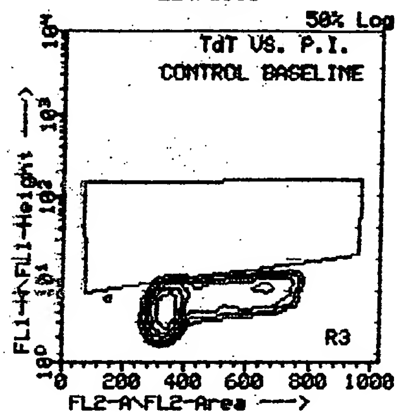
224FL001



224FL001



224FL001



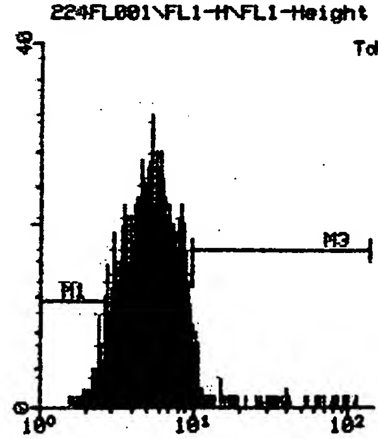
224FL001

----- Region Stats -----

File: 224FL001 Sample: FL5.12 CONTI
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2487

Rgn	Events	% Gated	% Total
1 R1	2487	100.00	82.90
2 R2	2487	100.00	82.90
3 R3	58	2.33	1.93

224FL001\FL1-H\FL1-Height



224FL001\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224FL001
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2487	100.00	32	5.23
1	1.00, 2.81	151	6.07	12	2.57
2	2.81, 9.82	2253	90.59	32	5.20
3	9.82, 136	97	3.90	8	11.14

Positive Contro

BECTON DICKINSON

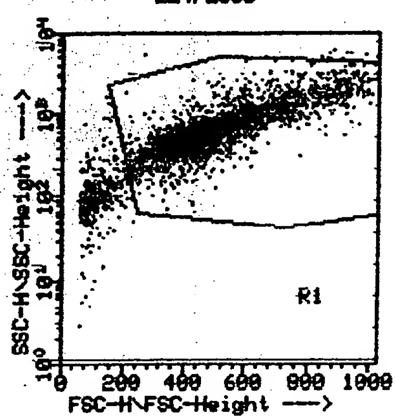
LYSYS II Ver 1.1

DATE:

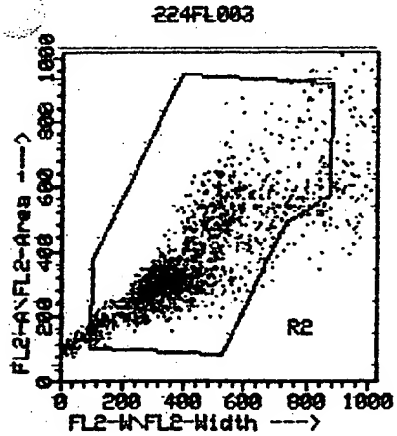
TIME: 15:14:19

SELECTED PREFERENCES: Arithmetic/Linear

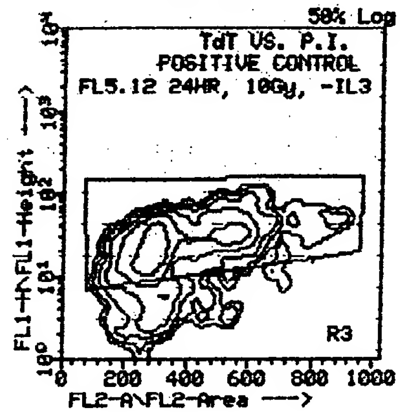
224FL003



224FL003



224FL003



224FL003

----- Region Stats -----

File: 224FL003 Sample: FL5.12 CONT

Date: Gate G2= R1ANDR2

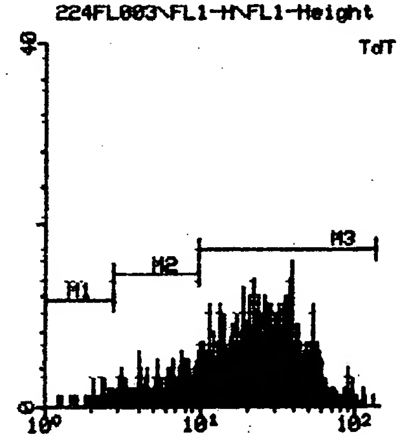
Selected Preference: Arithmetic/Lin

Parameters: FL2-A(LIN),FL1-H(LOG)

Total= 3000 Gated= 1625

Rgn	Events	% Gated	% Total
1 R1	1625	100.00	54.17
2 R2	1625	100.00	54.17
3 R3	1335	82.15	44.50

224FL003\FL1-H\FL1-Height



224FL003\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224FL003

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1625	100.00	16	21.87
1	1.00, 2.81	49	3.02	3	2.37
2	2.81, 9.82	274	16.86	6	6.13
3	9.82, 136	1307	80.43	16	26.42

THE UNIVERSITY OF CHICAGO
ANIMAL RESOURCES CENTER

REQUEST FOR TRANSFER OF ANIMAL OWNERSHIP

Requested transfer date _

We request the following animals be transferred:

SPECIES nude mice QUANTITY 56

CAGE CARD NUMBER(S)

AA113928 through AA113943 (16 cages)

REQUESTED TRANSFER FROM:

PRINCIPAL INVESTIGATOR ______

PROTOCOL ______ FAS ACCOUNT ______

PHONE ______ SIGNATURE ______

this animal(s) been used in research or teaching? YES NO

If yes how ?______

The number of unused animals transferred will be restored to the number available for ordering under this ACUP.

REQUESTED TRANSFER TO:

PRINCIPAL INVESTIGATOR HALLAHAN / SIBLEY

PROTOCOL ______ FAS ACCOUNT ______

PHONE 2-0294 SIGNATURE [signature]

Will this animal(s) be moved to a new housing site / room? YES NO

If yes where? CARLSON J-013

The number of animals transferred will be deducted from the number available for ordering under this ACUP.

ARC APPROVALS:

CLINICAL VETERINARIAN APPROVAL ______ DATE ______

Animals which have been used or which are to be moved to a different housing site require the approval of a clinical veterinarian.

BUSINESS OFFICE APPROVAL ______ DATE ______

ANSACTION DATE ______ GCS ENTRY BY______

(2/95)

For committee use:

ACUP No.: 58671B

ACUP Amendment–Supplemental Form A

Only the primary investigator of an ACUP is permitted to make changes to that ACUP. Please note that certain changes in an ACUP may affect other aspects of that ACUP, and should be reflected in this amendment. ACUPs with amendments require Institutional Animal Care and Use Committee (IACUC) review and approval. The IACUC reserves the right to determine whether proposed changes are substantive or not, and to request further information or a new ACUP application, as appropriate. When submitting an amendment, the Principal Investigator is required to review all of the details of the original ACUP and to assure the IACUC that all unamended details remain identical to the original ACUP. Please note that the amendment must be typed.

Investigator: Dennis Hallahan, M.D.

Department: Radiation Oncology

ACUP Number: 58671B

Original approval date:

The following changes are herein proposed for this protocol:

[ ] staff involved

Note: Include the number of years of work experience with the species used in this protocol, and confirm that each individual has reviewed the **University of Chicago Manual on Laboratory Animals Interim Guide.** If anyone listed has less than one year of relevant experience, please briefly describe how they will be or have been trained.

[ ] housing [X] procedure [ ] agents used

[ ] number of animals [ ] change in co-investigator

[ ] site outside centrally managed animal facilities to which live animals are taken and route of animal transport

[ ] response to questions raised during the IACUC-ARC review process [ ] other

Provide sufficient details, including a rationale for the proposed change, in narrative form to allow the committee to make a decision. Use additional pages as necessary and <u>submit revised pages of the original ACUP, as appropriate.</u>

Clarification of endpoints used in protocol #58671B:

1.) Tumor Size: The cutoff as mentioned in the protocol is "10% of body weight". Tumor measurements are typically performed twice weekly and tumor volumes are estimated by the formula **1/2(length x width x height)**. This is is derived from the equation for the volume of a sphere= $4\pi r^3/3 = 4r^3 = d^3/2 = (l \times w \times h)/2$. Since most nude mice weigh 18-25 grams, a tumor size of **2000 mm^3** = 2 cc = 2 grams is approximately 10% of body weight. This will be used as the cutoff for tumor size and can be easily verified by those monitoring the animals.

2.) Ulceration of tumors: When rapidly growing tumors outgrow their blood supply, they typically undergo necrosis in the central portion of the tumor. This creates a necrotic cavity which may heal completely over time if the tumor is cured. This situation is also seen in humans, most commonly in patients undergoing treatment for head and neck cancers. In our experience, this central necrosis is not tender in nude mice and has not predisposed the animals to infection, weight loss or loss of limb function. We continue to use these latter three conditions as criteria to sacrifice the animals.

Dennis Hallahan

Investigator

Date

This form must be submitted bearing the original signature of the investigator.

01 ACC–ARC 2/7/95

The University of Chicago
Departmental Purchase Order

Purchase Order Number
Z890627
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials controlled substances, vehicle rental or other restricted items.

Vendor Name
VWR Scientific
P.O. Box 66929
O'Hare AMF
Chicago | IL | 60666
City | State | Zip Code

Payment Terms ______ Delivery charge? ☐ Yes ☒ No
Telephone No. 1-800-932-5000 FAX No. ______

Ship To
THE UNIVERSITY OF CHICAGO   Dept. Code: ______
Radiation Oncology
Department
Greg Sibley, MD   6-03
Attn: Room
5830 S. Ellis Ave
Chicago | IL | 60637
City | State | Zip Code

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? ☐ No ☒ Yes
Sharon
Order placed with (name)   Date
Greg Sibley   2-0294
Order placed by (name)   Phone #

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
4	1	Vernier Calipers 19155-057	49.88	199.52
		VWR Acct # 77055-172		

D[illegible]ARTMENT COPY

NOT VALID IF TOTAL [illegible]XCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER

ORDER TOTAL ______

Form ZDPO 100 07/94

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 24129

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Greg Sibley, MD DATE:

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE:

FAS ACCOUNT: 2-73731-5100 P.I.: Hallen

VENDOR: FCRI PROTOCOL: 5861

REQUESTED DELIVERY DATE: PHONE: 2-0294

SPECIES: Mouse QUANTITY: 80

STRAIN: Athymic Nude SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

Mice to go to Carlson Biohazard Suite Room J-013

HOUSE AT: X CARLSON ___ WYLER ~~X~~ CLSC ___ FMI ___ OTHER

J-013

Apoptosis Assay - U87 Cells

Purpose - To see if apoptosis is seen in virally treated groups at earlier timepoints and to see if apoptosis is seen with TNF+RT at other time points

Methods - T-150 flasks of U87 cells were grown to ~ 80% confluence

			7^{AM} -10	1^{PM} -4	5^{pm} 0°	11^{pm} 6°	5^{pm} 24°	5^{pm} 48°	5^{pm} 72°	%A
	Control						Harvest			1.8
2	TNF+RT	6°		TNF	RT	Harvest				2.6
3	"	24°		TNF	RT		Harvest			10.
4		48°		TNF	RT			Harvest		5
5		72°		TNF	RT				Harvest	16.
6	10^7 899-6	6°	infect			Harvest				4
10		24°	infect				Harvest			13.
7	10^7 899-6+RT	6°	infect		RT	Harvest				5.6
11		24°	infect		RT		Harvest			25
8	10^8 899-6	6°	infect			Harvest				13.7
12		24°	infect				Harvest			11.
9	10^8 899-6+RT	6°	infect		RT	Harvest				8.
13		24°	infect		RT		Harvest			9

Infection - cells were infected in 6 ml of 199V media x 2°
then additional 14 cc of no FCS media was added

RT - Single fraction of 20 Gy

TNF - 10 ul of 0.01 mg/ml stock in 20 ml media

Control
⊖ TdT

BECTON DICKINSON

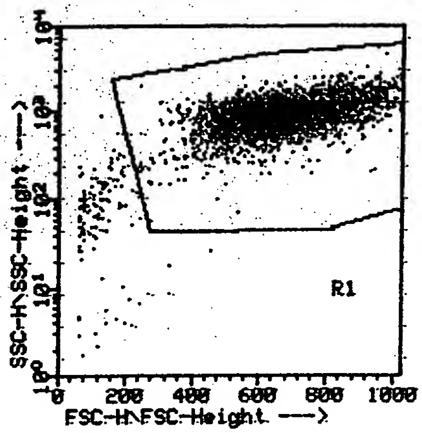
LYSYS II Ver. 1.1

DATE:

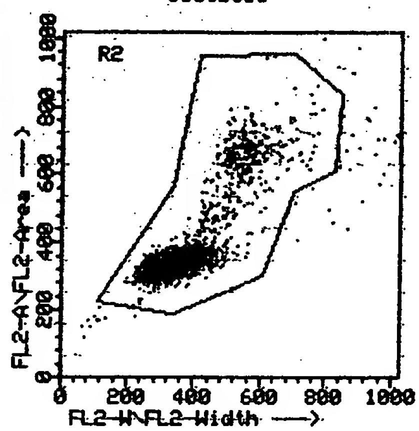
TIME: 15:03:12

SELECTED PREFERENCES: Arithmetic/Linear

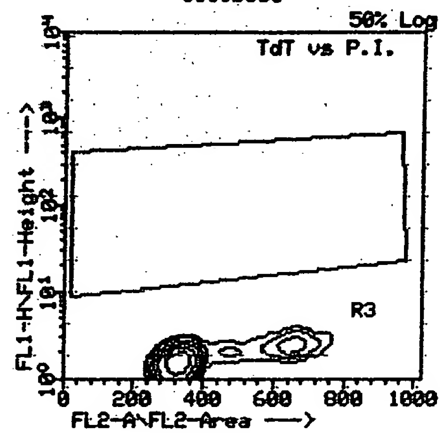
55SIB000



55SIB000



55SIB000



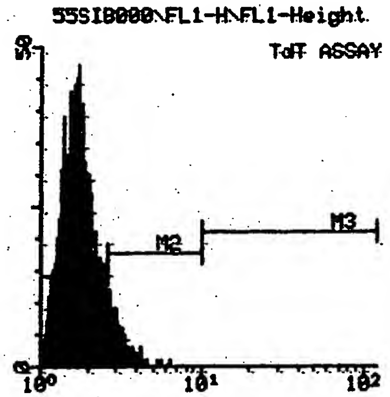
55SIB000

---- Region Stats ----

File: 55SIB000 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2399

Rgn	Events	% Gated	% Total
1 R1	2399	100.00	79.97
2 R2	2399	100.00	79.97
3 R3	0	0.00	0.00

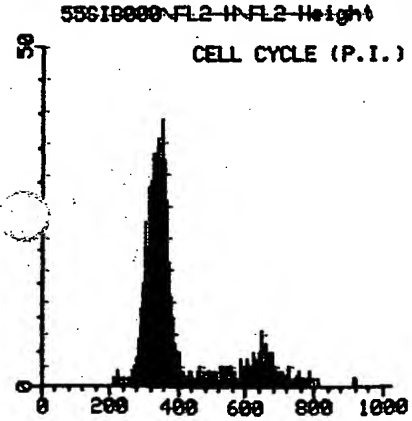
55SIB000\FL1-H\FL1-Height



55SIB000\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB000
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2399	100.00	47	1.70
1	1.00, 2.64	2202	91.79	47	1.67
2	2.64, 9.91	208	8.67	12	2.94
3	9.91, 118	0	0.00	0	--

55SIB000\FL2-H\FL2-Height



0

Control
+ TdT

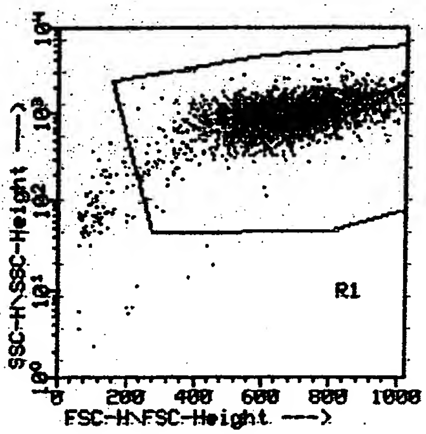
BECTON
DICKINSON

LYSYS II Ver 1.1

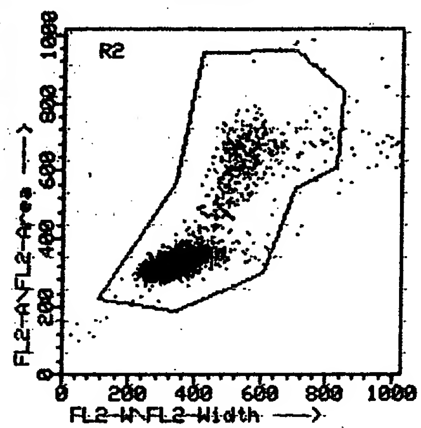
DATE: TIME: 15:04:24

SELECTED PREFERENCES: Arithmetic/Linear

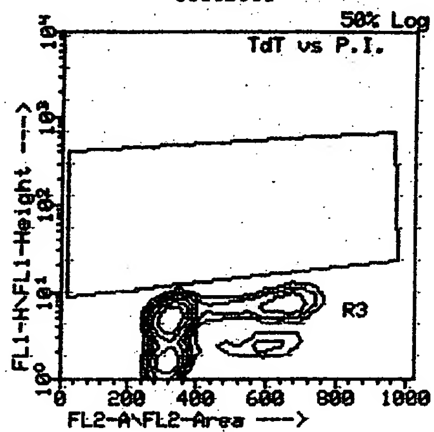
55SIB001



55SIB001



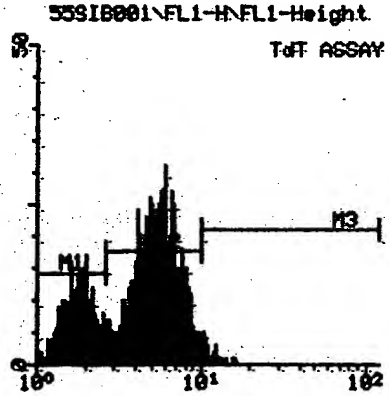
55SIB001



55SIB001
----- Region Stats -----
File: 55SIB001 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2399

Rgn	Events	% Gated	% Total
1 R1	2399	100.00	79.97
2 R2	2399	100.00	79.97
3 R3	1	0.04	0.03

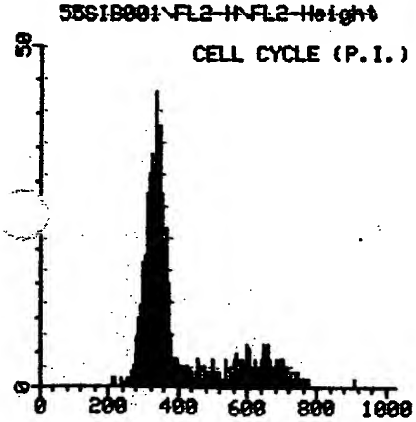
55SIB001\FL1-H\FL1-Height



55SIB001\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB001
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2399	100.00	31	4.74
1	1.00, 2.64	646	26.93	17	1.78
2	2.64, 9.91	1717	71.57	31	5.42
3	9.91, 118	44	1.83	5	18.89

55SIB001\FL2-H\FL2-Height



1.8

TNF+RT 6°

BECTON DICKINSON

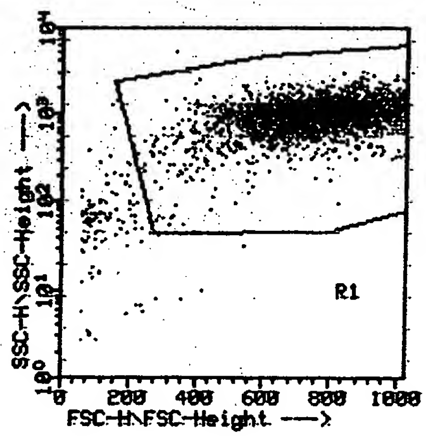
LYSYS II Ver 1.1

DATE:

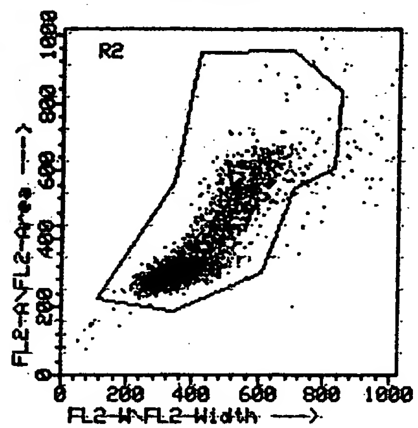
TIME: 15:05:22

SELECTED PREFERENCES: Arithmetic/Linear

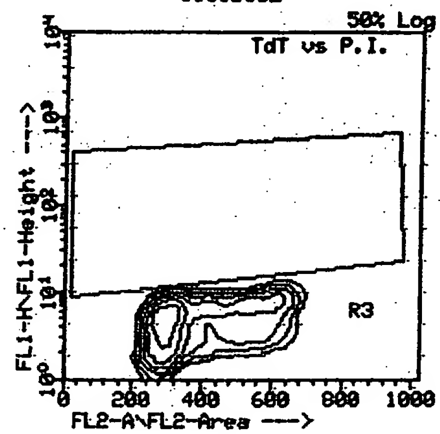
55SIB002



55SIB002



55SIB002



55SIB002

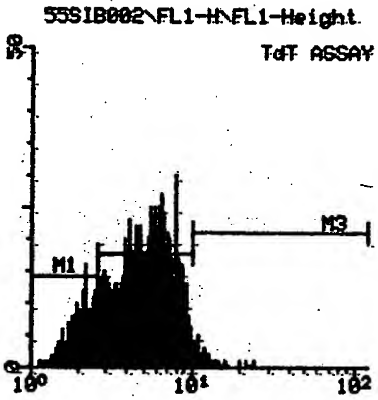
---- Region Stats ----

File: 55SIB002 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2450

Rgn	Events	% Gated	% Total	:
1 R1	2450	100.00	81.67	1:
2 R2	2450	100.00	81.67	1!
3 R3	4	0.16	0.13	1;

55SIB002\FL1-H\FL1-Height

TdT ASSAY



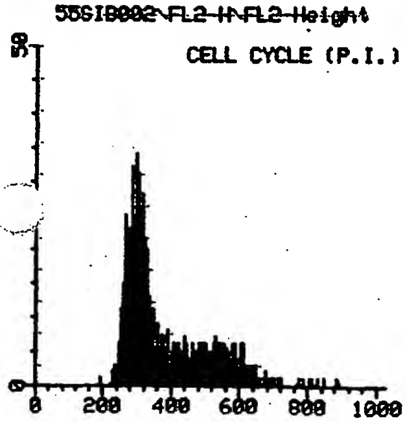
55SIB002\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB002
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2450	100.00	30	4.83
1	1.00, 2.64	400	16.33	16	2.15
2	2.64, 9.91	2003	81.76	30	5.28
3	9.91, 118	63	2.57	5	10.84

55SIB002\FL2-H\FL2-Height

CELL CYCLE (P.I.)



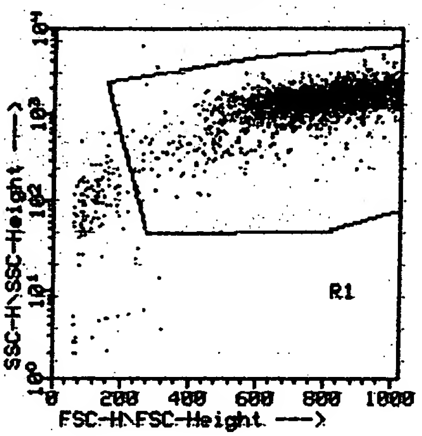
2.6

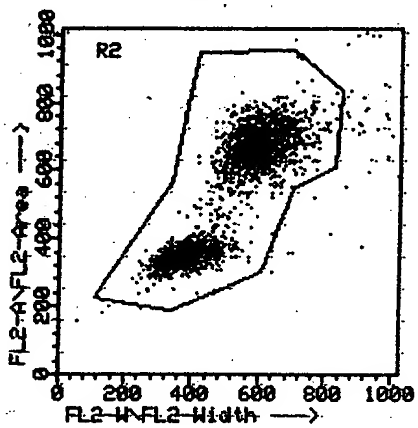
TNF+RT 24°

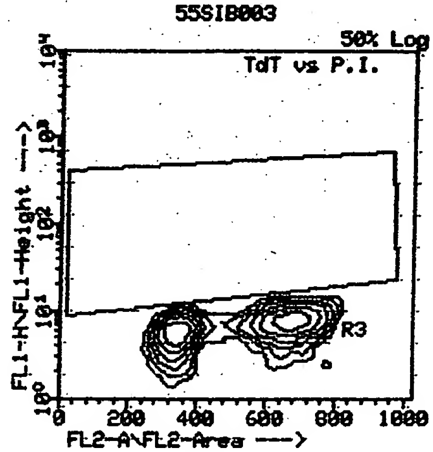
BECTON DICKINSON LYSYS II Ver 1.1

DATE: TIME: 15:06:21

SELECTED PREFERENCES: Arithmetic/Linear





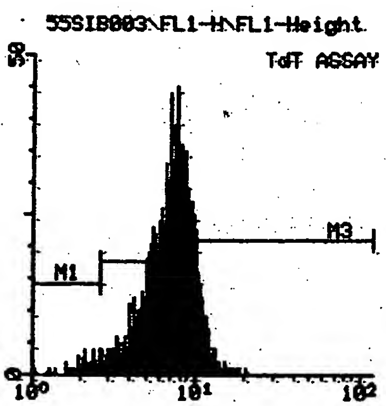


55SIB003

---- Region Stats ----

File: 55SIB003 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2437

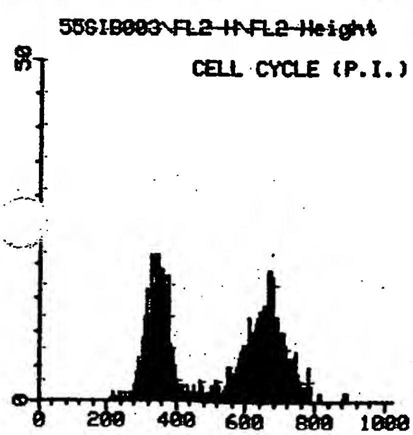
Rgn	Events	% Gated	% Total	:
1 R1	2437	100.00	81.23	2
2 R2	2437	100.00	81.23	1
3 R3	4	0.16	0.13	1



55SIB003\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB003
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2437	100.00	45	7.10
1	1.00, 2.64	56	2.30	4	2.19
2	2.64, 9.91	2148	88.14	45	6.92
3	9.91, 118	248	10.18	16	10.84

10.2

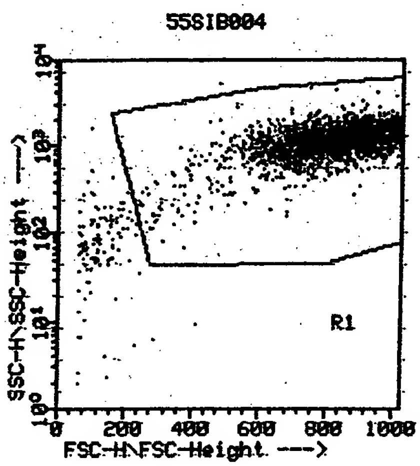


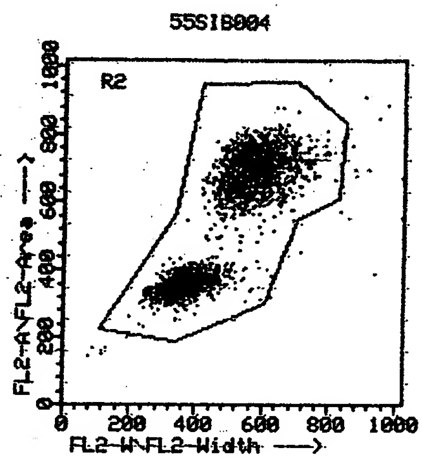
TNF+RT 480

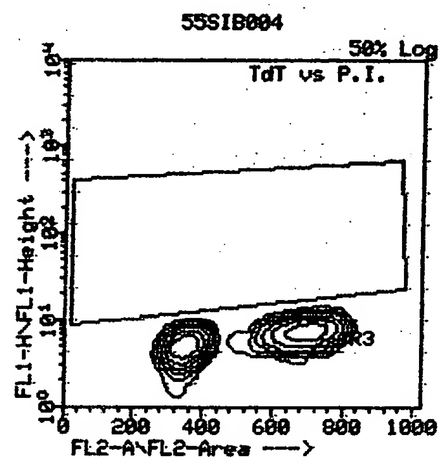
BECTON DICKINSON LYSYS II Ver 1.1

DATE: TIME: 15:07:20

SELECTED PREFERENCES: Arithmetic/Linear





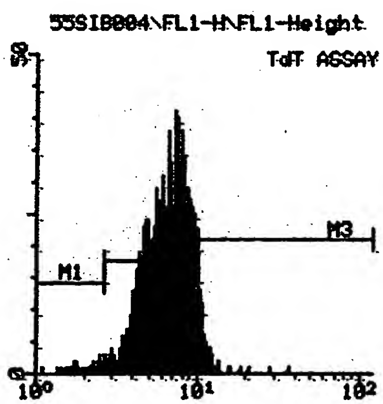


55SIB004

------ Region Stats ------

File: 55SIB004 Sample: SIBLEY APOP !
Date: . Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2479

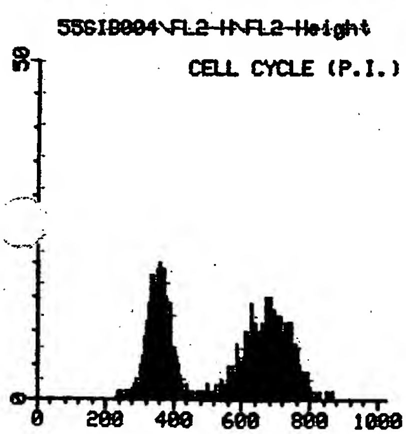
Rgn	Events	% Gated	% Total	:
1 R1	2479	100.00	82.63	2
2 R2	2479	100.00	82.63	1;
3 R3	4	0.16	0.13	1·



55SIB004\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB004
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2479	1[illegible].00	41	6.61
1	1.00, 2.64	35	41	3	2.09
2	2.64, 9.91	23[illegible]	[illegible].03	41	6.55
3	9.91, 118	[illegible]	[illegible]04	13	10.46



5

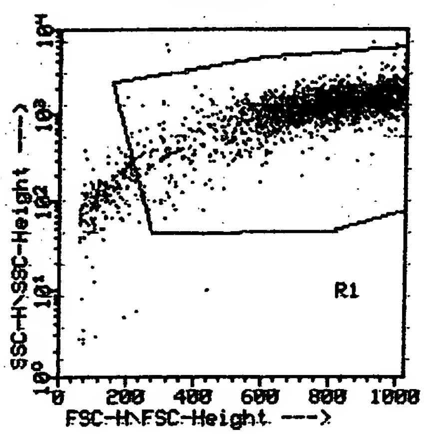
TNF+RT 72°

BECTON DICKINSON LYSYS II Ver 1.1

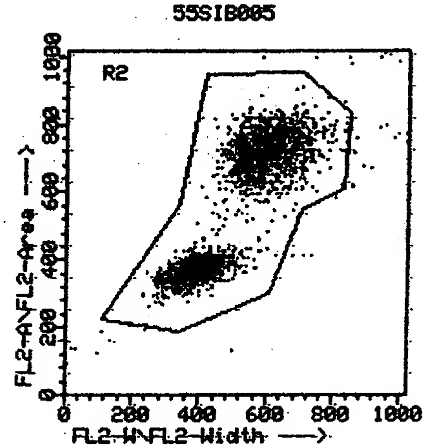
DATE: TIME: 15:08:20

SELECTED PREFERENCES: Arithmetic/Linear

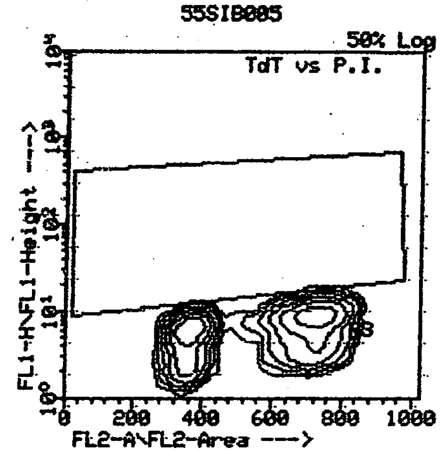
55SIB005



55SIB005



55SIB005



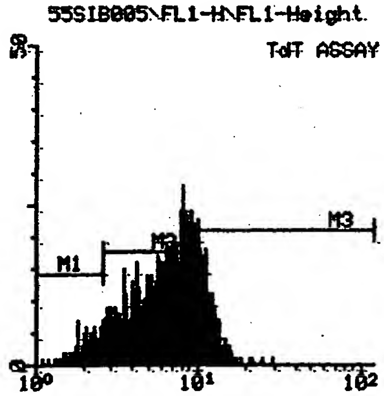
55SIB005

---- Region Stats ----

File: 55SIB005 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2162

Rgn	Events	% Gated	% Total	:
1 R1	2162	100.00	72.07	2
2 R2	2162	100.00	72.07	1
3 R3	7	0.32	0.23	1

55SIB005\FL1-H\FL1-Height

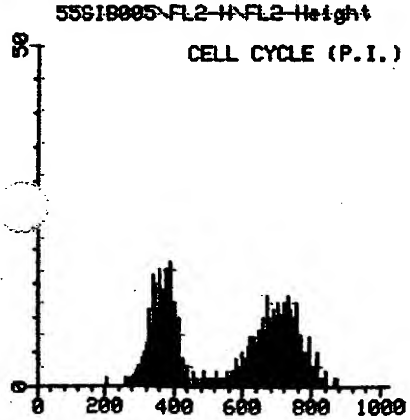


55SIB005\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB005
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2162	100.00	28	6.73
1	1.00, 2.64	167	7.72	7	2.19
2	2.64, 9.91	1656	76.60	28	6.38
3	9.91, 110	360	16.65	10	11.14

16.7

55SIB005\FL2-H\FL2-Height



2x10^6 899-6 · 6°

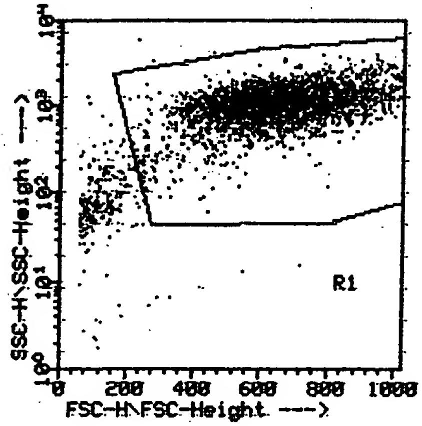
BECTON
DICKINSON

LYSYS II Ver 1.1

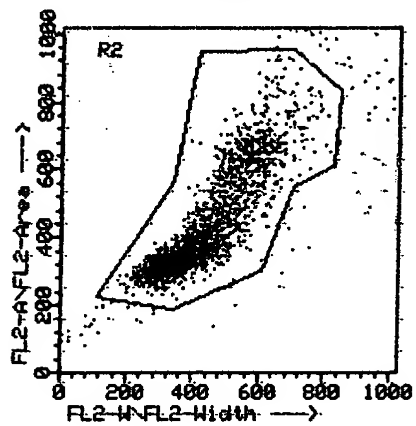
DATE: TIME: 15:09:18

SELECTED PREFERENCES: Arithmetic/Linear

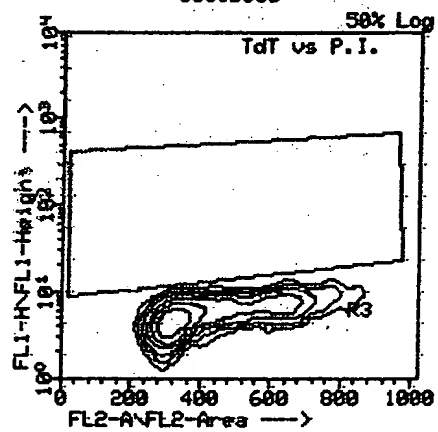
55SIB006



55SIB006



55SIB006



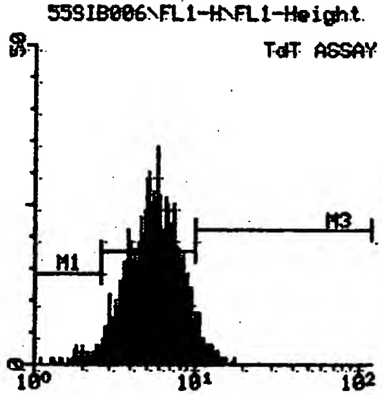
55SIB006

----- Region Stats -----

File: 55SIB006 Sample: SIBLEY APOP :
Date: : Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2146

Rgn	Events	% Gated	% Total	:
1 R1	2146	100.00	71.53	1:
2 R2	2146	100.00	71.53	1(
3 R3	2	0.09	0.07	:

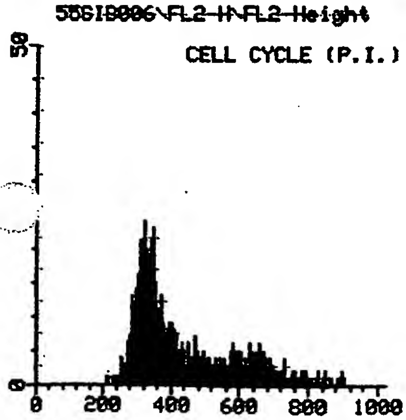
55SIB006\FL1-H\FL1-Height



55SIB006\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB006
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2146	100.00	34	5.57
1	1.00, 2.64	56	2.61	3	2.11
2	2.64, 9.91	2006	93.48	34	5.52
3	9.91, 118	93	4.33	8	10.65

55SIB006\FL2-H\FL2-Height



4

2x10^6 898-6 +RT 6°

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DICKINSON

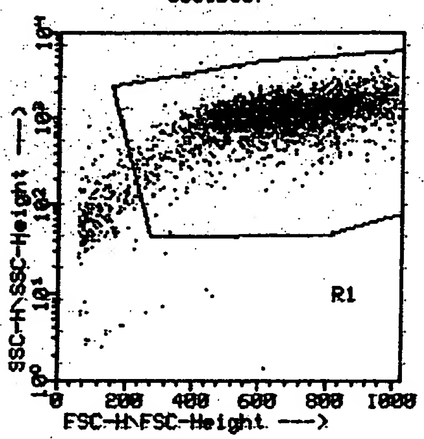
LYSYS II Ver 1.1

DATE:

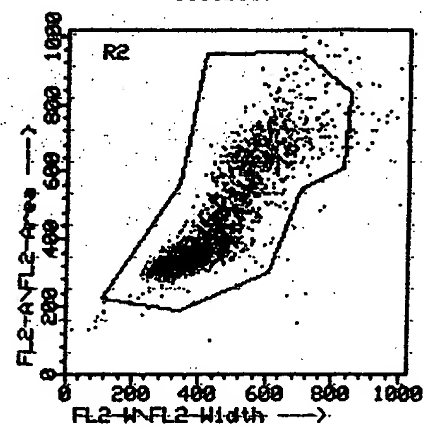
TIME: 15:10:15

SELECTED PREFERENCES: Arithmetic/Linear

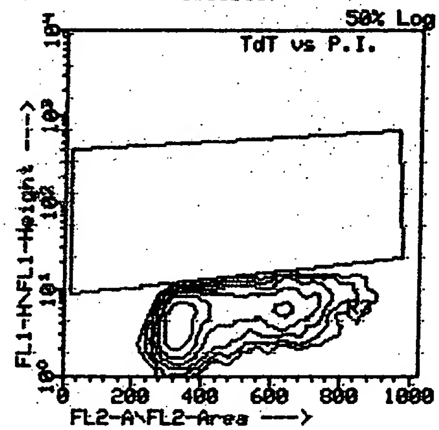
55SIB007



55SIB007



55SIB007



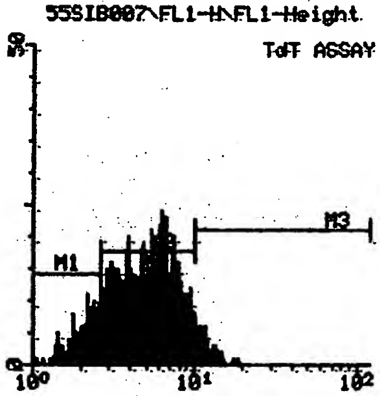
55SIB007

----- Region Stats -----

File: 55SIB007 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2128

Rgn	Events	% Gated	% Total	:
1 R1	2128	100.00	70.93	1
2 R2	2128	100.00	70.93	1
3 R3	2	0.09	0.07	1

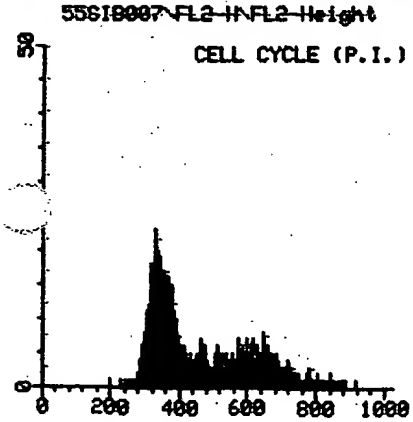
55SIB007\FL1-H\FL1-Height



55SIB007\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB007
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2128	100.00	24	4.87
1	1.00, 2.64	292	13.72	14	2.17
2	2.64, 9.91	1727	81.16	24	5.23
3	9.91, 118	120	5.64	8	11.24

55SIB007\FL2-H\FL2-Height



5.6

2×10^{7} 889-6- 6°

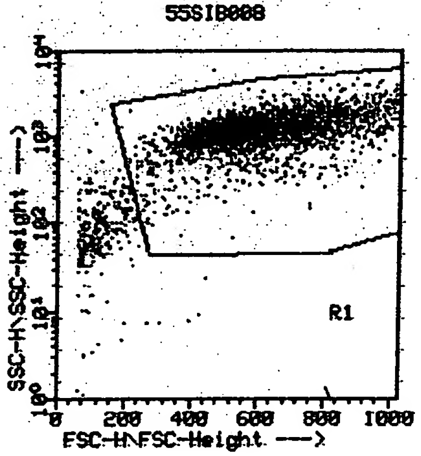
BECTON
DICKINSON

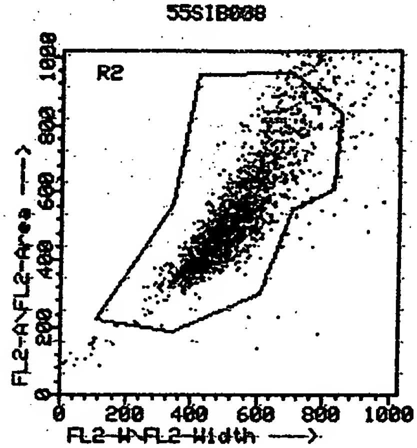
LYSYS II Ver 1.1

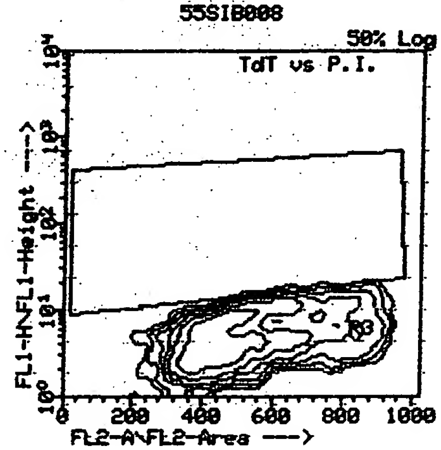
DATE:

TIME: 15:11:13

SELECTED PREFERENCES: Arithmetic/Linear







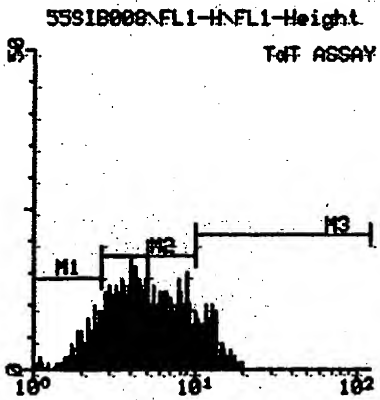
55SIB008

----- Region Stats -----

File: 55SIB008 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1748

Rgn	Events	% Gated	% Total	:
1 R1	1748	100.00	58.27	1.
2 R2	1748	100.00	58.27	1:
3 R3	2	0.11	0.07	1

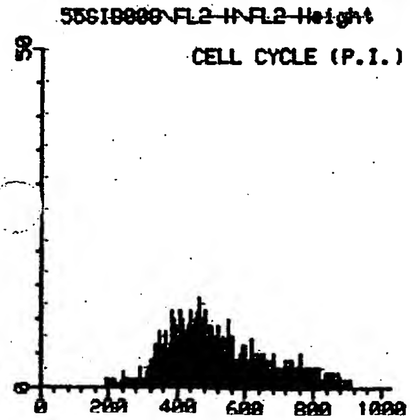
55SIB008\FL1-H\FL1-Height



55SIB008\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB008
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1748	100.00	20	4.78
1	1.00, 2.64	225	12.87	10	2.23
2	2.64, 9.91	1298	74.26	20	4.74
3	9.91, 118	240	13.73	10	11.92

55SIB008\FL2-H\FL2-Height



13.7

2x10 7 899-6+RT 6°

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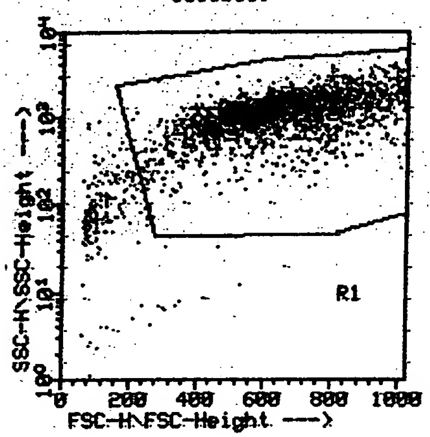
LYSYS II Ver 1.1

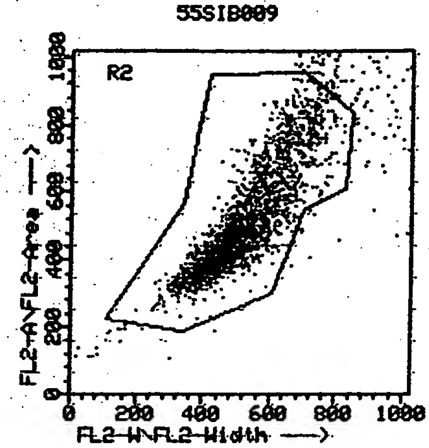
DATE: TIME: 15:12:14

SELECTED PREFERENCES: Arithmetic/Linear

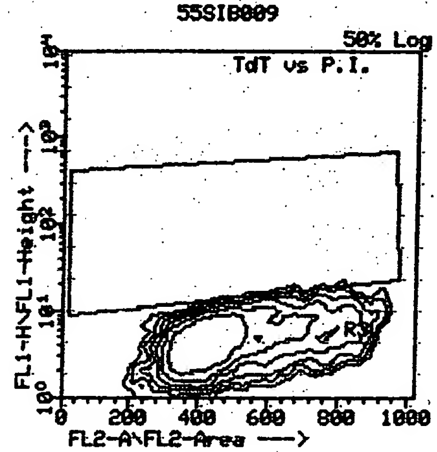
55SIB009



55SIB009



55SIB009



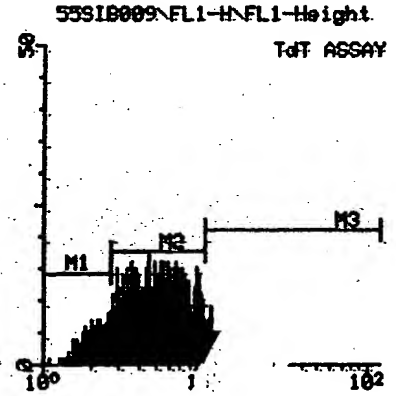
55SIB009

------ Region Stats ------

File: 55SIB009 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1817

Rgn	Events	% Gated	% Total	
1 R1	1817	100.00	60.57	1!
2 R2	1817	100.00	60.57	1:
3 R3	6	0.33	0.20	1:

55SIB009\FL1-H\FL1-Height

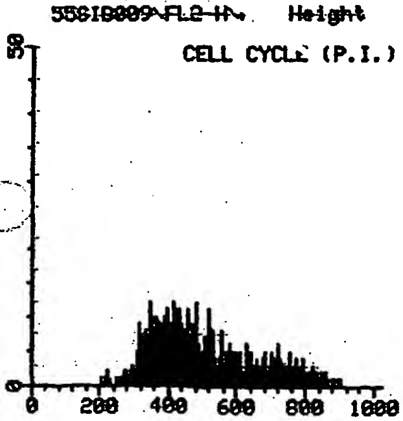


55SIB009\FL1-H\FL1-Height
---- Arithmetic Histogram Statistics for 55SIB009
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1817	100.00	17	5.00
1	1.00, 2.64	236	12.99	8	2.13
2	2.64, 9.91	1435	78.98	17	5.19
3	9.91, 118	156	8.59	9	11.44

8.6

55SIB009\FL2-H\ Height



2×10^{6} 888-6 24°

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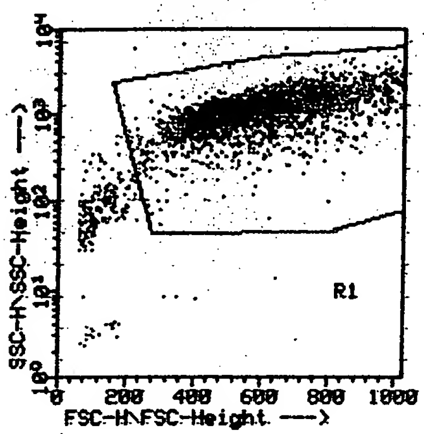
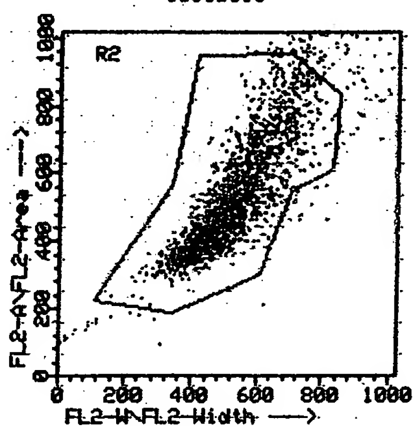
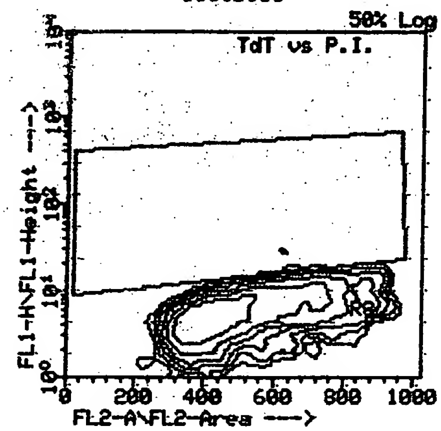
DATE: TIME: 15:13:13

SELECTED PREFERENCES: Arithmetic/Linear







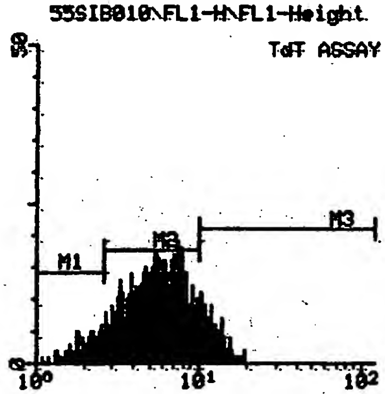
55SIB010

---- Region Stats ----

File: 55SIB010 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1748

Rgn	Events	% Gated	% Total	:
1 R1	1748	100.00	58.27	1:
2 R2	1748	100.00	58.27	1:
3 R3	1	0.06	0.03	1:

55SIB010\FL1-H\FL1-Height

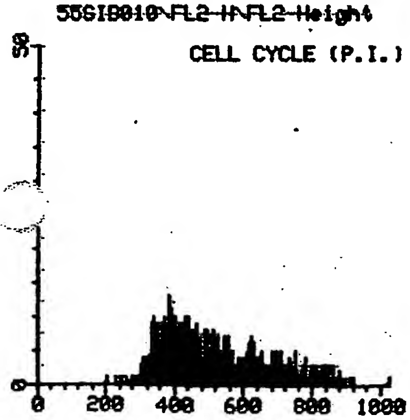


55SIB010\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB010
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1748	100.00	19	5.62
1	1.00, 2.64	157	8.98	6	2.15
2	2.64, 9.91	1369	78.32	19	5.52
3	9.91, 110	235	13.44	11	11.76

55SIB010\FL2-H\FL2-Height



13.4

2×10^{6} 859-6 + RT 24°

BECTON DICKINSON

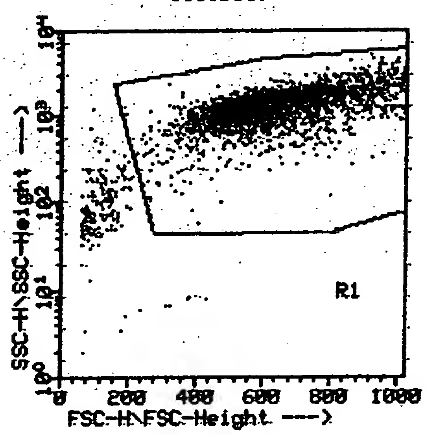
LYSYS II Ver 1.1

DATE:

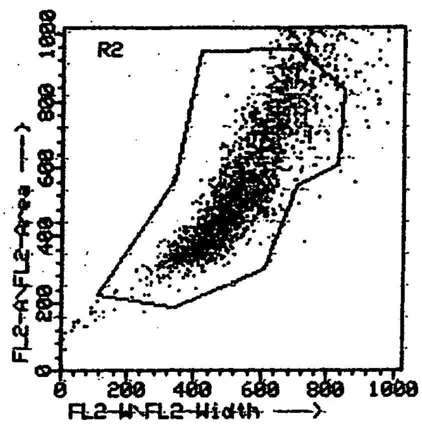
TIME: 15:14:11

SELECTED PREFERENCES: Arithmetic/Linear

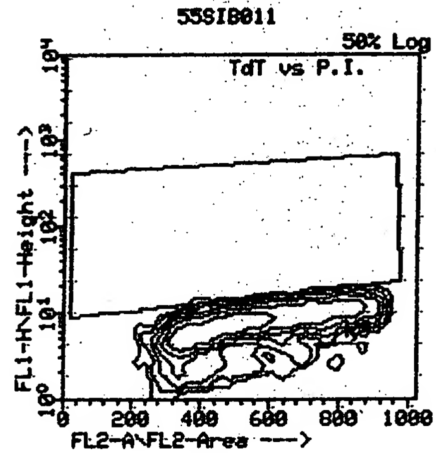
55SIB011



55SIB011



55SIB011



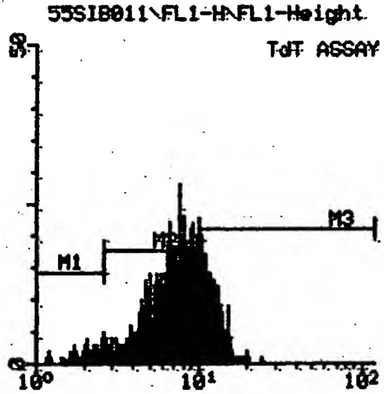
55SIB011

---- Region Stats ----

File: 55SIB011 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line.
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1815

Rgn	Events	% Gated	% Total	:
1 R1	1815	100.00	60.50	1
2 R2	1815	100.00	60.50	1:
3 R3	5	0.28	0.17	1

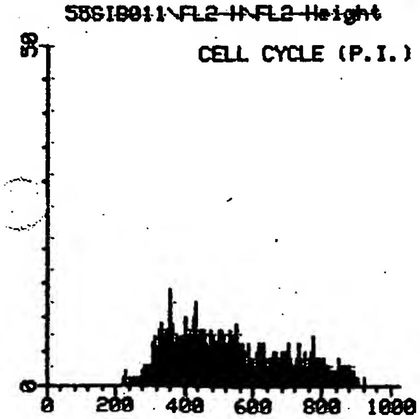
55SIB011\FL1-H\FL1-Height



55SIB011\FL1-H\FL1-Height
---- Arithmetic Histogram Statistics for 55SIB011
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1815	100.00	28	7.64
1	1.00, 2.64	95	5.23	5	2.09
2	2.64, 9.91	1290	71.07	28	6.92
3	9.91, 118	454	25.01	20	11.55

55SIB011\FL2-H\FL2-Height



25

$2x10^7$ 888-6   24°

BECTON DICKINSON

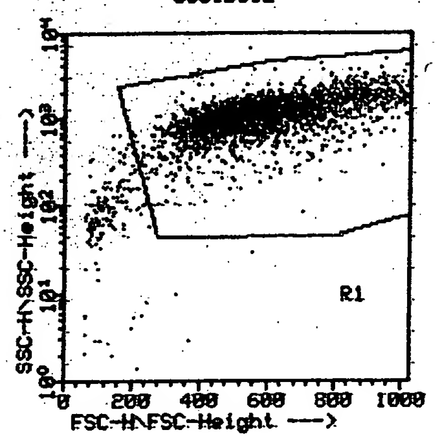
LYSYS II Ver 1.1

DATE:

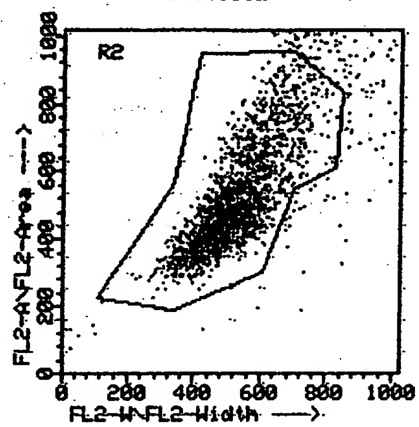
TIME: 15:15:11

SELECTED PREFERENCES: Arithmetic/Linear

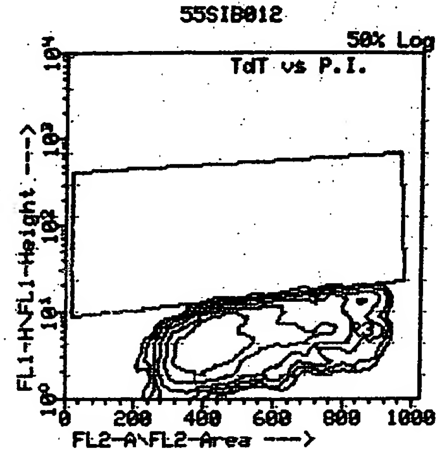
55SIB012



55SIB012



55SIB012



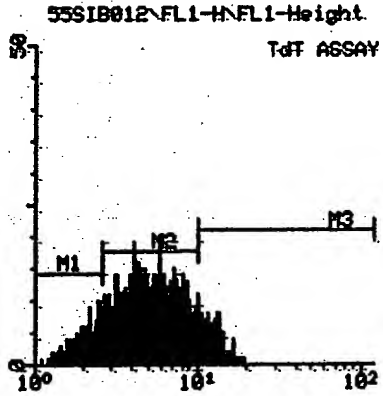
55SIB012

—— Region Stats ——

File: 55SIB012 Sample: SIBLEY APOP !
Date: : Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line.
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1795

Rgn	Events	% Gated	% Total	:
1 R1	1795	100.00	59.83	1:
2 R2	1795	100.00	59.83	1;
3 R3	3	0.17	0.10	1i

55SIB012\FL1-H\FL1-Height



55SIB012\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB012

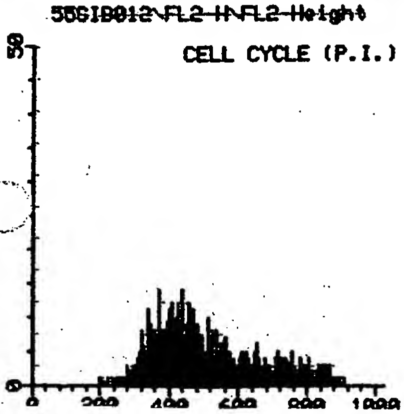
Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height   Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1795	100.00	19	4.91
1	1.00, 2.64	269	14.99	10	2.11
2	2.64, 9.91	1339	74.60	19	5.09
3	9.91, 118	205	11.42	11	11.65

11.4

55SIB012\FL2-H\FL2-Height



2x10⁷ 8E4-6 +RT 24

BECTON DICKINSON

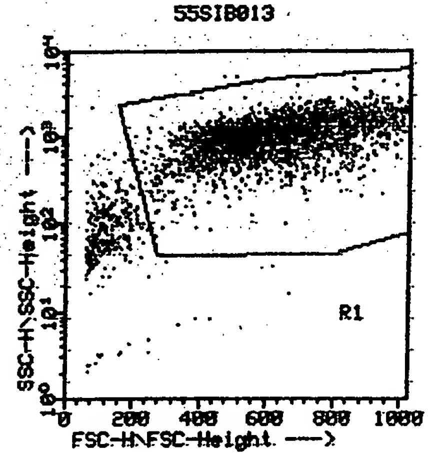
LYSYS II Ver 1.1

DATE:

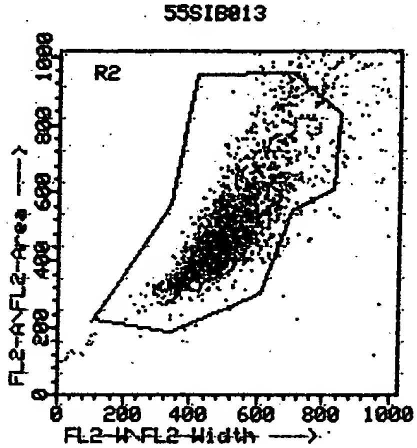
TIME: 15:16:10

SELECTED PREFERENCES: Arithmetic/Linear

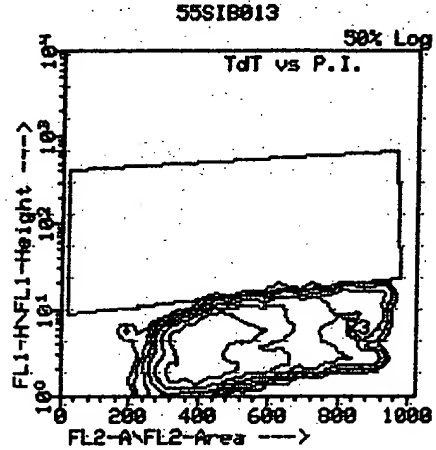
55SIB013



55SIB013



55SIB013



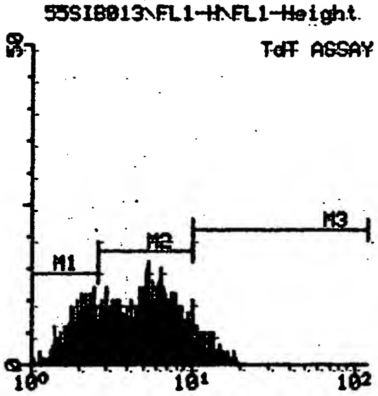
55SIB013

----- Region Stats -----

File: 55SIB013 Sample: SIBLEY APOP !
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1622

Rgn	Events	% Gated	% Total	:
1 R1	1622	100.00	54.07	1.
2 R2	1622	100.00	54.07	1:
3 R3	4	0.25	0.13	1:

55SIB013\FL1-H\FL1-Height



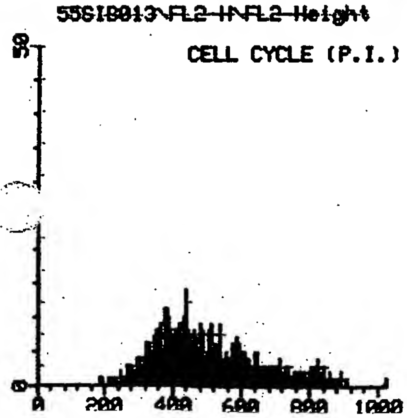
55SIB013\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB013
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1622	100.00	16	4.49
1	1.00, 2.64	416	25.65	12	2.07
2	2.64, 9.91	1074	66.21	16	5.19
3	9.91, 118	146	9.00	10	11.88

9

55SIB013\FL2-H\FL2-Height



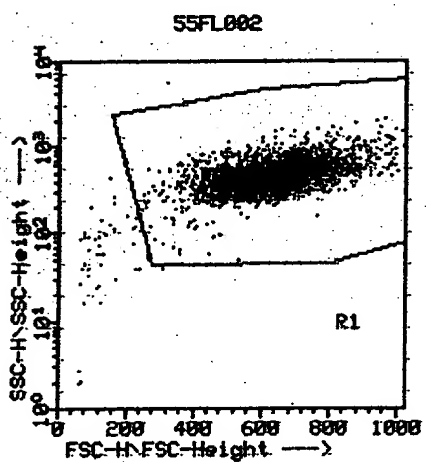
Pos Control
⊖ TdT

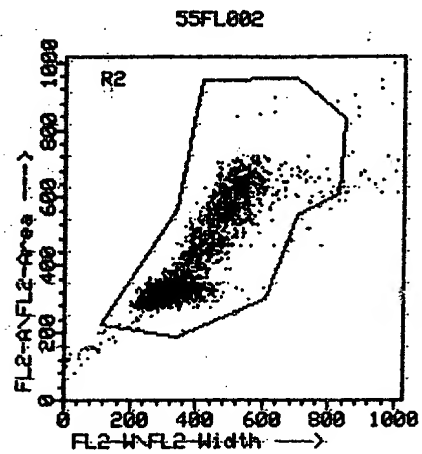
BECTON
DICKINSON

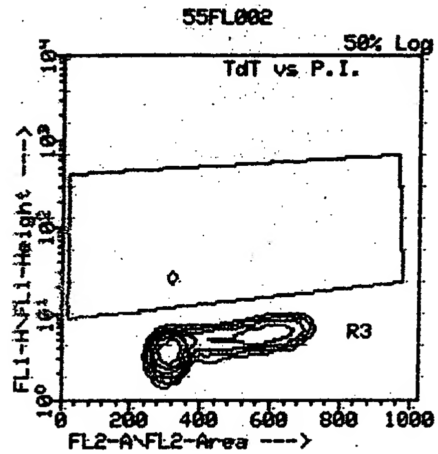
LYSYS II Ver 1.1

DATE: TIME: 15:19:50

SELECTED PREFERENCES: Arithmetic/Linear







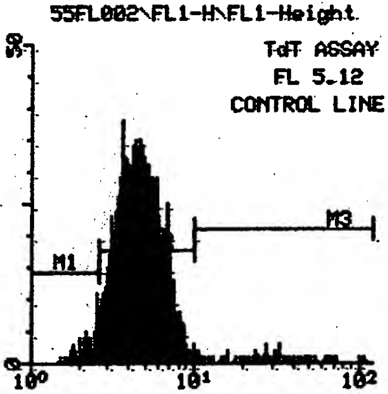
55FL002

------ Region Stats ------

File: 55FL002 Sample: SIBLEY APOP 5.
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line.
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2684

Rgn	Events	% Gated	% Total
1 R1	2684	100.00	89.47
2 R2	2684	100.00	89.47
3 R3	58	2.16	1.93

55FL002\FL1-H\FL1-Height

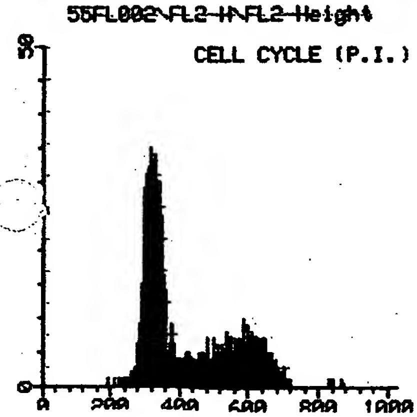


55FL002\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55FL002 -
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2684	100.00	38	4.49
1	1.00, 2.64	121	4.51	11	2.35
2	2.64, 9.91	2501	93.18	38	4.53
3	9.91, 118	68	2.53	3	27.14

2.5

55FL002\FL2-H\FL2-Height



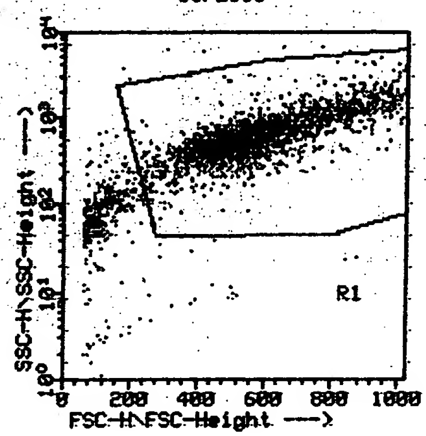
Positive control ⊕ TdT

BECTON DICKINSON LYSYS II Ver 1.1

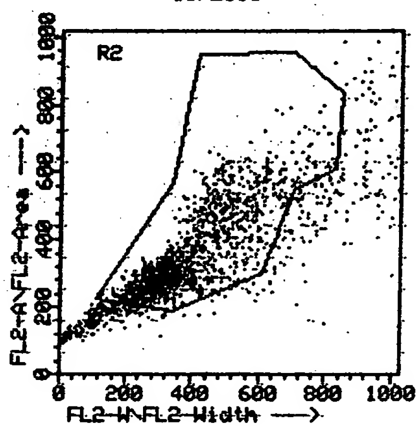
DATE: TIME: 15:21:40

SELECTED PREFERENCES: Arithmetic/Linear

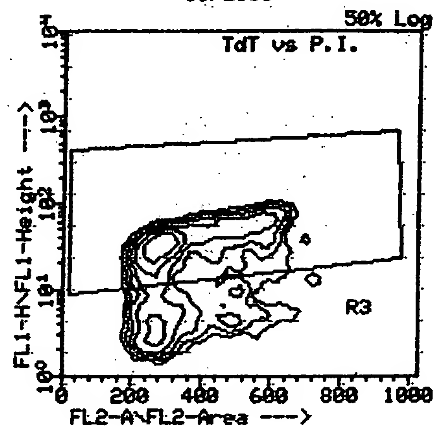
55FL003



55FL003



55FL003



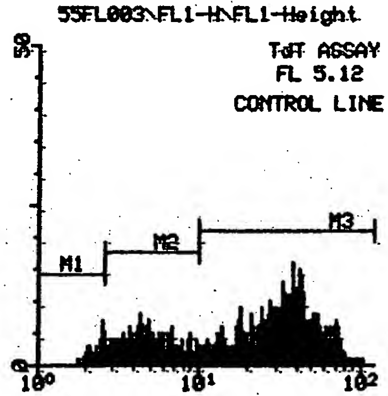
55FL003

------ Region Stats ------

File: 55FL003 Sample: SIBLEY APOP 5.
Date: Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1439

Rgn	Events	% Gated	% Total
1 R1	1439	100.00	47.97
2 R2	1439	100.00	47.97
3 R3	962	66.85	32.07

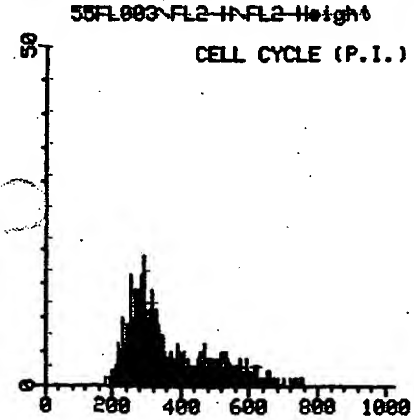
55FL003\FL1-H\FL1-Height



55FL003\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55FL003 -
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1439	100.00	16	25.71
1	1.00, 2.64	56	3.89	7	2.44
2	2.64, 9.91	374	25.99	8	4.96
3	9.91, 118	1012	70.33	16	34.29

55FL003\FL2-H\FL2-Height



70.3

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0							
2075	AA105283	10^7 899-6	0	0	0							
1713	AA105283	10^7 899-6	0	0	0							
2054	AA111331	10^7 899-6	0	0	0							
2057	AA111332	10^7 899-6										
1709	AA104549	10^7 3616										
1723	AA104549	10^7 3616	0	0	0							
2041	AA111334	10^7 3616										
2064	AA111334	10^7 3616										
1859	AA108972	RT Alone	12.5	11.5	9							
1861	AA108972	RT Alone										
1862	AA108972	RT Alone	6	5	4							
1892	AA108964	RT Alone	6	4.5	3							
1863	AA108971	10^7 3616+RT	4.5	4	3.5							
1864	AA108971	10^7 3616+RT	14	9.5	7							
1865	AA108971	10^7 3616+RT	4.5	3.5	1							
1866	AA108971	10^7 3616+RT	4	5	1							
1883	AA108966	10^7 3616+RT	0	0	0							
1884	AA108966	10^7 3616+RT	1	1	1							
1886	AA108966	10^7 3616+RT	0	0	0							
2074	AA108966	10^7 3616+RT	9.5	8.5	6							
2032	AA111326	10^7 3616+RT	6	5	4.5							
2033	AA111326	10^7 3616+RT	5	4	3							
2035	AA111326	10^7 3616+RT	8	7	4.5							
2072	AA111326	10^7 3616+RT	0	0	0							
2036	AA111327	10^7 3616+RT	7	6	3.5							
2037	AA111327	10^7 3616+RT	4	3	1.5							
2039	AA111327	10^7 3616+RT	9	8	5							
2073	AA111327	10^7 3616+RT	4	5	1.5							
1871	AA108969	10^7 899-6+RT	3.5	3.5	2							
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT	5	7	4.5							
1874	AA108969	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0							
188[illegible]	AA108967	10^7 899-6+RT	0	0	0							
18[illegible]	AA108967	10^7 899-6+RT	2.5	2	1							
2[illegible]	AA111329	10^7 899-6+RT	4.5	3.5	2.5							
20[illegible]	AA111329	10^7 899-6+RT	0	0	0							
2046	AA111329	10^7 899-6+RT	4.5	5	2.5							
2047	AA111329	10^7 899-6+RT										
2048	AA111330	10^7 899-6+RT	2.5	2.5	1.5							
2049	AA111330	10^7 899-6+RT	16	13.5	11.5							
2050	AA111330	10^7 899-6+RT	2.5	3.5	2							
2051	AA111330	10^7 899-6+RT	9	8	4.5							

2204			11.5	11	9							
2189			10	11	9							
2190			12	10	8							
2191			13	9	8							
2192			12	10.5	9							
2201			6.5	6.5	4.5							
2202			7	7.5	5							
2203			7	7.5	4.5							

LOGSHEETS

1710	AA105283	10^7 899-6	<10 d35	0	23.7		
2075	AA105283	10^7 899-6	<10 d42	0	23.0		
1713	AA105283	10^7 899-6	<10 d14	0	22.2		
2054	AA111331	10^7 899-6	<10 d49	0	23.4		
1709	AA104549	10^7 3616	<10 d31				
1723	"	"		0	24.3		
1859	AA108972	RT Alone		12.5x11x10	24.0		
1862	AA108972	RT Alone		5.5.3	25.1		
1892	AA108964	RT Alone		5x4x2	24.4		
2189	AA113941	RT Alone		10x11.5x8	22.0		
2190	AA113941	RT Alone		11.5x8.5x7	18.2		
2191	AA113941	RT Alone		12.5x8x6.5	18.6		
2192	AA113941	RT Alone		12x10x8	22.0		
2201	AA113944	RT Alone		8.5x10x8	22.6		
2202	AA113944	RT Alone		8.5x10x7	21.3		
2203	AA113944	RT Alone		8x7x6	24.3		
2204	AA113944	RT Alone		12.5x12.5x10	23.1		
1863	AA108971	10^7 3616+RT		5.5x4x3	24.7		
1864	AA108971	10^7 3616+RT		13.5x10x6.5	27.6		
1865	AA108971	10^7 3616+RT	<10 d38	4x3x1	27.0		
1866	AA108971	10^7 3616+RT	<10 d31	0	26.3		
1883	AA108966	10^7 3616+RT	<10 d24	0	22.6		
1884	AA108966	10^7 3616+RT	<10 d56	2x2x1.5	23.9		
1886	AA108966	10^7 3616+RT	<10 d31	0	21.5		
2074	AA108966	10^7 3616+RT		9.5x9x6	17.0		
2032	AA111326	10^7 3616+RT		6x7.5x4.5	22.1		
2033	AA111326	10^7 3616+RT		4x4x2.5	25.0		
2035	AA111326	10^7 3616+RT		7x8.5x5	22.2		
2072	AA111326	10^7 3616+RT	<10 d21	~~000~~ 0	24.7		
2036	AA111327	10^7 3616+RT		~~6x2.5x4.5~~	~~22.1~~		
2037	AA111327	10^7 3616+RT	<10 d52	3x2x1	25.3		
2039	AA111327	10^7 3616+RT		8.5x9x5.5	17.6		
2073	AA111327	10^7 3616+RT		4x4x1	20.6		
				6.5x6x3	22.8		
1871	AA108969	10^7 899-6+RT	<10 d66	3.5x3.5x2	24.0		
1873	AA108969	10^7 899-6+RT		6x6x4	24.6		
1874	AA108969	10^7 899-6+RT	<10 d52	0	27.8		
1880	AA108967	10^7 899-6+RT	<10 d14	0	21.7		
1881	AA108967	10^7 899-6+RT	<10 d42	0	26.4		
1882	AA108967	10^7 899-6+RT	<10 d24	0	24.0		
2044	AA111329	10^7 899-6+RT		4.5x4x3	23.8		
2045	AA111329	10^7 899-6+RT	<10 d21	0	27.3		
2046	AA111329	10^7 899-6+RT		4.5x4x2	25.0		
2048	AA111330	10^7 899-6+RT	<10 d38	2x2.5x1	15.9		
2049	AA111330	10^7 899-6+RT		18.5x14x13.5	25.2		
2050	AA111330	10^7 899-6+RT	<10 d31	3x2x1.5	23.9		
2051	AA111330	10^7 899-6+RT		7x8x3.5	24.0		

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
~~1709~~	AA104549	10^7 3616	<10 d31				
1723				0			
1859	AA108972	RT Alone	11.5 x10 x 10.5				
1862	AA108972	RT Alone	4.5 x5 x3.5				
1892	AA108964	RT Alone	4.5 x3.5 x3				
2189	AA113941	RT Alone	10.5 x11 x9				
2190	AA113941	RT Alone	11.5 x8 x8.5				
2191	AA113941	RT Alone	10.5 x7 x6.5				
2192	AA113941	RT Alone	11 x12 x9				
2201	AA113944	RT Alone	10 x 9.5 x9				
2202	AA113944	RT Alone	7.5 x7 x6.5				
2203	AA113944	RT Alone	8.5 x10 x7				
2204	AA113944	RT Alone	11.5 x11 x9				
1863	AA108971	10^7 3616+RT		4 x5 x3			
1864	AA108971	10^7 3616+RT		15 x12 x8			
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	2 x2 x1.5			
1886	AA108966	10^7 3616+RT	<10 d31	~~3x4x7~~ 0			
2074	AA108966	10^7 3616+RT		8 x9 x4			
2032	AA111326	10^7 3616+RT	~~5x7x4.5~~	5 x7 x4.5			
2033	AA111326	10^7 3616+RT		4 x4 x 2.5			
2035	AA111326	10^7 3616+RT		8 x6 x5			
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		5.5 x6.5 x3			
2037	AA111327	10^7 3616+RT	<10 d52	3 x2.5 x1.5			
2039	AA111327	10^7 3616+RT		10 x8 x6			
2073	AA111327	10^7 3616+RT		4 x3 x1			
1871	AA108969	10^7 899-6+RT	<10 d66	3 x4 x2.5			
1873	AA108969	10^7 899-6+RT		6 x5 x3			
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		5 x4 x4			
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		4 x3.5 x2.5			
2048	AA111330	10^7 899-6+RT	<10 d38	1.5 x1 x1			
2049	AA111330	10^7 899-6+RT		18 x13 x11			
2050	AA111330	10^7 899-6+RT	<10 d31	3 x2 x1			
2051	AA111330	10^7 899-6+RT		6 x8 x4.5			

OVER

1710	AA105283	10^7 899-6	<10 d35	0	0	0	23.8
2075	AA105283	10^7 899-6	<10 d42	0	0	0	22
1713	AA105283	10^7 899-6	<10 d14	0	0	0	20.8
2054	AA111331	10^7 899-6	<10 d49	0	0	0	22.4
~~1709~~	AA104549	10^7 3616	<10 d31	0	0	0	25.3
23							
1859	AA108972	RT Alone		12	14	10	24.5
1862	AA108972	RT Alone		5.5	9	3	24.4
1892	AA108964	RT Alone		5	5	3	23.5
2189	AA113941	RT Alone		11	12	8	22.0
2190	AA113941	RT Alone		12	9.5	7	17.8
2191	AA113941	RT Alone		12	8.5	6	19.2
2192	AA113941	RT Alone		12	11	9	20.3
2201	AA113944	RT Alone		11	12	9	22.2
2202	AA113944	RT Alone		6.5	6.5	5.5	24.2
2203	AA113944	RT Alone		11.5	10	8.5	20.4
2204	AA113944	RT Alone		12	11.5	7	19.9
1863	AA108971	10^7 3616+RT		5	4	2	23.3
1864	AA108971	10^7 3616+RT		14	13	7	25.4
1865	AA108971	10^7 3616+RT	<10 d38	0	0	0	25.6
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	25.2
1883	AA108966	10^7 3616+RT	<10 d24	0	0	0	22.1
1884	AA108966	10^7 3616+RT	<10 d56	0	0	0	23.9
1886	AA108966	10^7 3616+RT	<10 d31	0	0	0	21.4
2074	AA108966	10^7 3616+RT		10	9.5	4	16.8
2032	AA111326	10^7 3616+RT		8	7	5.5	23.2
2033	AA111326	10^7 3616+RT		4.5	4	1.5	25.1
2035	AA111326	10^7 3616+RT		0	0	0	24.4
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	4	23.3
2036	AA111327	10^7 3616+RT		6	6.5	3	23.8
2037	AA111327	10^7 3616+RT	<10 d52	5	3.5	1.5	25.2
2039	AA111327	10^7 3616+RT		11	9	6	16.8
2073	AA111327	10^7 3616+RT		0	0	0	20.9
1871	AA108969	10^7 899-6+RT	<10 d66	7	6	2.5	23.9
1873	AA108969	10^7 899-6+RT		5	5	3	23.3
1874	AA108969	10^7 899-6+RT	<10 d52	0	0	0	27.9
1880	AA108967	10^7 899-6+RT	<10 d14	0	0	0	21.2
1881	AA108967	10^7 899-6+RT	<10 d42	0	0	0	25.1
1882	AA108967	10^7 899-6+RT	<10 d24	6	5	1.5	23.9
2044	AA111329	10^7 899-6+RT		7	6	4.5	24.3
2045	AA111329	10^7 899-6+RT	<10 d21	3.5	3	2	25.4
2046	AA111329	10^7 899-6+RT		8	0	0	25.4
2048	AA111330	10^7 899-6+RT	<10 d38	0	0	0	16.2
2049	AA111330	10^7 899-6+RT		21.5	15	14	25.3
2050	AA111330	10^7 899-6+RT	<10 d31	0	0	0	23.8
2051	AA111330	10^7 899-6+RT		8	10	5.5	24.6

113945 L 2089 8.5 9.5 7.5 21.3
R 2090 9 8.5 7.5 22
LR

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
23							
1859	AA108972	RT Alone		12.5	14	10.5	
1862	AA108972	RT Alone		5	5	3.5	
1892	AA108964	RT Alone		4.5	5	2.5	
2189	AA113941	RT Alone		11	11.5	8	
2190	AA113941	RT Alone		9	12	6.5	
2191	AA113941	RT Alone		8	11	4	
2192	AA113941	RT Alone		11	11	7	
2201	AA113944	RT Alone		11	11	7	
2202	AA113944	RT Alone		7	7	5	
2203	AA113944	RT Alone		11.5	12	9	
2204	AA113944	RT Alone		12	12	8.5	
1863	AA108971	10^7 3616+RT		4	5	2	
1864	AA108971	10^7 3616+RT		14	11.5	6	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	
1883	AA108966	10^7 3616+RT	<10 d24	0	0		
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	~~13~~ 0	~~10~~ 0	~~6~~ 0	
2074	AA108966	10^7 3616+RT		13	10	6	
2032	AA111326	10^7 3616+RT		6.5	8	6	
2033	AA111326	10^7 3616+RT		4	3.5	2	
2035	AA111326	10^7 3616+RT		0			
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	6	
2036	AA111327	10^7 3616+RT		6	7	2.5	
2037	AA111327	10^7 3616+RT	<10 d52	4	4	2	
2039	AA111327	10^7 3616+RT		12	10	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	6	5.5	2.5	
1873	AA108969	10^7 899-6+RT		5	5	3	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		6.5	6	4	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3.5	3.5	2	
2048	AA111330	10^7 899-6+RT	<10 d38	3.5	3	1	
2049	AA111330	10^7 899-6+RT		22000			
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		7.5	10	5	

113945 2206 9.5 9 7.5 CONTROLS
2207 11 11.5 9

AVER

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			23.2
1713	AA105283	10^7 899-6	<10 d14	0			22.3
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1859	AA108972	RT Alone		14	13.5	10	25
1862	AA108972	RT Alone		5	5	4	24.4
1892	AA108964	RT Alone		5	4	3	23.8
2189	AA113941	RT Alone		10.5	10	8	20.9
died drawn ~~2190~~ 2198	AA113941	RT Alone		11	8	6.5	18.2
~~2191~~	AA113941	RT Alone					
2192	AA113941	RT Alone		11	9	7	22.2
2201	AA113944	RT Alone		10.5	10.5	8	20.3
2202	AA113944	RT Alone		6.5	5	4	24
2203	AA113944	RT Alone		10	10	7	20.6
2204	AA113944	RT Alone		11	11.5	8.5	21.1
1863	AA108971	10^7 3616+RT		4	3	3	23.8
1864	AA108971	10^7 3616+RT		13.5	12	7	25.3
1865	AA108971	10^7 3616+RT	<10 d38	0			26.4
1866	AA108971	10^7 3616+RT	<10 d31	0			26.2
1883	AA108966	10^7 3616+RT	<10 d24	0			22.2
1884	AA108966	10^7 3616+RT	<10 d56	2	1	1	24.2
1886	AA108966	10^7 3616+RT	<10 d31	0			26.2
2074	AA108966	10^7 3616+RT		16	11	7	27
2032	AA111326	10^7 3616+RT		7.5	5.5	4.5	23.3
2033	AA111326	10^7 3616+RT		4	3	2.5	25.7
2035	AA111326	10^7 3616+RT		9.5	7	6	23.9
2072	AA111326	10^7 3616+RT	<10 d21	0			25.5
2036	AA111327	10^7 3616+RT		6	5	4	26.6
2037	AA111327	10^7 3616+RT	<10 d52	3	3	1.5	26
2039	AA111327	10^7 3616+RT		10	8.5	6	18
2073	AA111327	10^7 3616+RT		0			21.6
1871	AA108969	10^7 899-6+RT	<10 d66	5	4	2	25.4
1873	AA108969	10^7 899-6+RT		7	5	4	24.6
1874	AA108969	10^7 899-6+RT	<10 d52	0			28.3
1880	AA108967	10^7 899-6+RT	[illegible]0 d14	0			22.0
1881	AA108967	10^7 899-6+R[illegible]	[illegible]0 d42	6	5	3.5	26.6
1882	AA108967	10^7 899-6[illegible]	[illegible]0 d24	0			24.2
2044	AA111329	10^7 899-6+R[illegible]		5	6.5	4.5	25.8
2045	AA111329	10^7 899-6+RT	<[illegible]0 d21	0			18.7
2046	AA111329	10^7 899-6+RT		3.5	3	2	24.9
2048	AA111330	10^7 899-6+RT	<10 d38	0			15.9
~~2049~~	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	5	3	2	25.6
2051	AA111330	10^7 899-6+RT		8.5	10.5	7.5	24.7

IN VIVO TNF PRODUCTION

Group	Mouse	Time	Tum weight	Vol Buffer	[TNF]	ΣTNF/tumor	TNF/mg-tumor
2x10^7 PFU 899-6	2370	3d	.45				
"	2371	3d	.26				
"	2372	3d	.37				
"	2373	3d	.44				
2x10^7 899-6	2374 ~~2386~~	10d	.13				
"	2375 ~~2387~~	10d	.54				
"	2376 ~~2388~~	10d	.61				
"	2377 ~~2389~~	10d	.23				
Control	2386	10d	2.41				
"	2387	10d	.35				
"	2388	10d	.47				
"	~~2389~~	~~10d~~					
2x10^7 3616	2390	3d	0.44				
"	2391	3d	1.23				
"	2392	10d	.24				
"	2393	10d	.69				

INDUCTION- in vivo

Mouse	Treatment	Tumor Wt	Vol Buffer	Reading	[TNF]pg/ml	[TNF]/tumor	[TNF]/n
2370	3d 899-6	0.45	1	0.679	331	480	1.0661
2371	3d 899-6	0.26	1	0.708	348	439	1.687
2372	3d 899-6	0.37	1	1.326	747	1023	2.7641
2373	3d 899-6	0.44	1	1.42	811	1168	2.6553
2374	10d 899-6	0.13	1	0.352	149	168	1.2938
2375	10d 899-6	0.54	1	0.444	197	304	0.5629
2376	10d 899-6	0.61	1	0.274	110	177	0.2897
2377	10d 899-6	0.23	1	0.323	134	165	0.717
2386	10d Control	2.41	1	2.021	1246	4249	1.7632
2387	10d Control	0.35	1	1.823	1099	1484	4.2401
2388	10d Control	0.47	1	2.498	1612	2370	5.0426
2390	3d 3616	0.44	1	0.37	158	228	0.5176
2391	3d 3616	1.23	1	0.515	236	527	0.4286
2392	10d 3616	0.24	1	0.374	160	199	0.8279
2393	10d 3616	0.69	1	0.756	377	637	0.9234

$$529.72 \times X^{1.2758}$$

$$y = .005863 * X^{.8188391}$$

Curve Fit-Plot © 1990 JCA

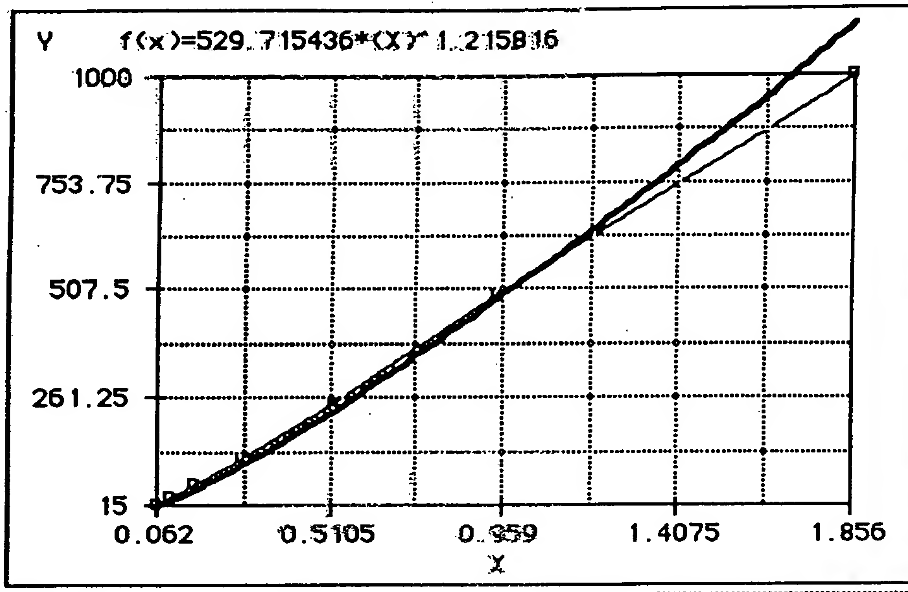
Modif./Inv. DATA:

X	Y
0.062	15.13
0.095	31.13
0.158	62.5
0.282	125
0.518	250
0.943	500
1.856	1000

• EXPONENTIAL FIT •

Y= 529.7154365*(X)^1.215816

Coef. of Regression= 0.995013 For 7 Data Pairs



INITIALS Y.K.
SUBJECT • Untilted ; [I: X<>Y]
DATE
CPU Time: 13 Secs.

Sikey

IMMUNOSTAINING & HISTOLOGY - U87

Group	Mouse	Time	Prep
2×10^7 PFU 899-6	2362	3d	formalin & RNA
"	2363	3d	formalin & RNA
"	2364	3d	frozen & RNA
"	2365	3d	frozen & RNA
2×10^7 PFU 3616	2382	3d	formalin & RNA
"	2383	3d	frozen & RNA
"	2384	10d	
"	2385	10d	
2×10^7 PFU 899-6	2366	10d	
"	2367	10d	
"	2368	10d	
"	2369	10d	
Control	2378	3d	formalin & RNA
"	2379	3d	frozen & RNA
"	2380	10d	EM, formalin, frozen
"	2381	10d	
	2389	10d	

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
~~1709~~	AA104549	10^7 3616	<10 d31	0			
1723							
1859	AA108972	RT Alone		13	13	11	
1862	AA108972	RT Alone		4.5	4	3	
1892	AA108964	RT Alone		4	4.5	2.5	
2189	AA113941	RT Alone		7	9.5	5.5	
2190	AA113941	RT Alone		9.5	10.5	7	
~~2191~~	~~AA11~~3941	RT Alone					
2192	AA113941	RT Alone		9	10	6.5	
2201	AA113944	RT Alone		10	11	8.5	
2202	AA113944	RT Alone		5.5	4	2.5	
2203	AA113944	RT Alone		9	10	7.5	
2204	AA113944	RT Alone		11	11.5	7.5	
1863	AA108971	10^7 3616+RT		3.5	3	3	
1864	AA108971	10^7 3616+RT		13	12	7.5	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	0			
2074	AA108966	10^7 3616+RT		10	14	6	
2032	AA111326	10^7 3616+RT		6.5	7	5	
2033	AA111326	10^7 3616+RT		4	3	2	
2035	AA111326	10^7 3616+RT		7	8.5	5	
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		6	4.5	3.5	
2037	AA111327	10^7 3616+RT	<10 d52	0			
2039	AA111327	10^7 3616+RT		8	9	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	0			
1873	AA108969	10^7 899-6+RT		6	5	3.5	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	6	7.5	4	
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		8	6.5	5.5	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3	4.5	1.5	
2048	AA111330	10^7 899-6+RT	<10 d38	0			
2049	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		13	9	9	

Control				
2354	15.5	14.5	11.5	23.5
55	20	15.5	12	21.2
56	15.5	11	8.5	23.8
57	13	16	10	21.0

3616+RT				
2032	8	6.5	7	23.9
33	3	3.5	2	25.8
2072	0			24.7
35	9	8	6	23.8

1	2	3	4	5
RT				
2342	14	14 6		20.9
43	12.5	10.5 8		22.3
44	10	10 8		20.6
45	12	10 7		21.2
RT				
2350	14	11.5 8	21.7	
51	9.5	7 6	22.7	
52	10	9 7	23.6	
53	14	9 8	20.6	
RT				
2189	9	9.5 7	20.6	
90	7	6 4	18.4	
92	9.5	9 5.5	24.1	
RT				
2201	10	10 9	19.3	
02	2.5	2.5 1	26.6	
03	11.5	11 6	20.0	
04	12	11 7.5	23.7	
RT				
1859	12	13 11.5	26.3	
1892	4.5	3 2.5	24.3	
1862	5	4.5 4.5	25.1	
RT				
2346	13.5	10.5 10	19.2	
47	12	10 7.5	23.0	
48	16	10 9	18.6	
49	9	11.5 7	22	
CONTROL				
2206	17.5	20 16.5	24.9	
07	13	14.5 11.5	24.9	
CONTROL				
2358	16	15.5 14	20.9	
59	16	15.5 11.5	20.7	
60	17.5	12 11	23.4	
61	13	16 10	22	

6	7	8		9
3616+RT				
2036	5.5	4.5	4	25.1
37	0			26.5
2073	0			21.3
39	8	7	4	21.2
3616+RT				
1883	0			21.6
84	0			23.9
2074	15.5	10	7	16.9
86	0			21.3
3616+RT				
1863	4	4	3.5	24.2
64	13	11	7	22.6
65	0			26.3
66	0			25.3
3616				
1723	0			
899-6				
2054	0			23.2
2075	0			24.2
1713	0			22
1710	0			24.4
899+RT				
1880	0			21.4
81	8	5	4	27
82	0			24
899+RT				
2048	0			14.6
50	0			25.3
51	14	10	9	25.1

EFFICIENCY LINE® 22-206 AMPAD

~~RT~~ Control
2354 17 17.5 11
55 22 17.5 11.5
56 16 14.5 8
57 16.5 ~~8~~13 9

3616 + RT
2032 8 8.5 6
33 4 3 2.5
2072 [redacted] 0
35 9 7.5 6

1	2	3	4	5	6	7	8	9

RT
2342 15.5 13.5 9.5
2343 12 9 7
2344 13 11 7.5
2345 7.5 10 7.5

RT 2350 17 10 8.5
2351 11 8 7
2352 10 8 7
2353 14 9.5 7

RT
2189 10 10 7
2190 6 6 3.5
2192 9.5 10 5

RT 2201 9 11 8
2202 0
03 9.5 9.5 5
04 11 11.5 7

RT
[redacted]

RT
2346 14 12 8.5
2347 12 11 8
2348 15 10 9
2349 11.5 10 7

CONTROL
2358 19.5 18 14
59 18.5 17.5 12
60 15 18 12
61 15.5 17.5 12

CONTROL
2206 20 22 18
2207 14.5 14.5 13.5

3616 + RT
[redacted] 5 4 3
[redacted] 12.5 10.5 7
[redacted] 0
[redacted] 0

3616 + RT
[redacted] 0
[redacted] 2 1 1
?sac ⇒ 2074 10 14 5.5
[redacted] 0

3616 + RT
2036 6 5 4
37 0
2073 0
2039 8 7 4

899-6 + RT
?sac ⇒ 2048 0
2050 6 4 1
2051 12 15 9

899 + RT
[redacted] 0
[redacted] 7 9.5 6
[redacted] 0

899 ~~+RT~~
2054 0
2075 0
~~173 0~~
~~170 0~~

899-6 + RT
2044 8 8.5 7
45 dead
46 0

The University of Chicago Departmental Purchase Order

Purchase Order Number
Z904416
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
Phar Mingen
10975 Torreyana Rd
San Diego, CA 92121
City State Zip Code
Payment Terms ______ Delivery charge? ☒ Yes ☐ No
Telephone No. 1-800-848-6227 FAX No. 619-792-5238

Ship To
THE UNIVERSITY OF CHICAGO Dept. Code: ______
Department: Dept Radiation Oncology
Attn: Greg Sibley, MD Room: 6-03
5830 S. Ellis Ave
City: Chicago State: IL Zip Code: 60637

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? ☐ No ☒ Yes
Order placed with (name): Daren Date: ______
Order placed by (name): Greg Sibley Phone #: 2-0294

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1	18641-D Purified Mouse Anti-human TNFα	$250	
		(Backordered 1-2 wks)	$ 20	

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

ORDER TOTAL $270

Form ZDPO 100 07/94

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Purchase Order Number
Z904417
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
VECTOR LABORATORIES
30 INGOLD RD
BURLINGAME, CA 94010
City | State | Zip Code
Payment Terms ____ Delivery charge? ☐ Yes ☐ No
Telephone No. 800-227-6666 FAX No. 415-697-0339

Ship To
THE UNIVERSITY OF CHICAGO
Dept. Code: ____
Dept Radiation Oncology
Department
Greg Sibley MD 6-03
Room
5830 S. Ellis Ave
Chicago IL 60637
City | State | Zip Code

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship to must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ____ Payroll No. ____
Print Name ____ Ext. ____
Account Code ____ Date ____

Order placed by phone? ☐ No ☒ Yes
Virginia
Order placed with (name) ____ Date ____
Greg Sibley 2-0294
Order placed by (name) ____ Phone #

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1Kit	PK-4002 Peroxidase vectastain ABC - Mouse IgG	$185.00	
			6.25	

D[illegible]ARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED

ORDER TOTAL $191.5

Form ZDPO 100 07/94

J-013

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207 L	AA113945	Control		18	16	11		25.4
2354	AA117027	Control ϕ		22	19	9		23.3
2356	AA117027	Control R		18	16.5	7		23.7
2357	AA117027	Control LR		17	14	10		19.6
2359	AA117028	Control L		22	18	11		20.5
2360	AA117028	Control R	18	[illegible]	18	12		24.6
2361	AA117028	Control LR		21	20	11		24.8
1710 LR	AA105283	10^7 899-6	<10 d35	ϕ	ϕ	ϕ		24.7
2075 ϕ	AA105283	10^7 899-6	<10 d42	ϕ	ϕ	ϕ		24.5
1713 R	AA105283	10^7 899-6	<10 d14	ϕ	ϕ	ϕ		21.5
2054 RR	AA~~111331~~	10^7 899-6	<10 d49	ϕ	ϕ	ϕ		22.5
	105283							
1723	AA104549	10^7 3616	<10 d28 face	ϕ	ϕ	ϕ		26.5
1859	AA108972	RT Alone ϕ		15	14	10		24.8
1862	AA108972	RT Alone LR		5	5	3		25.1
1892	AA108~~964~~ 72	RT Alone R		4	4	2		24.1
2189 ϕ	AA113941	RT Alone		10	10	4.5		24.0
2190 L	AA113941	RT Alone		5	5	2.5		20.9
2192 LR	AA113941	RT Alone		9	9	3		25.2
2201 ϕ	AA113944	RT Alone		8	8	3		19.6
2202 L	AA113944	RT Alone		ϕ	ϕ	ϕ		27.0
2203 R	AA113944	RT Alone		11	11	4.5		20.8
2204 LR	AA113944	RT Alone		12	11.5	7		26.6
2342 ϕ	AA117024	RT Alone		14	12	5		22.1
2343 L	AA117024	RT Alone		11.5	9	3.5		22.5
2344 R	AA117024	RT Alone		12.5	9	5		22.4
2345 LR	AA117024	RT Alone		15	8.5	5		22.9
2346 ϕ	AA117025	RT Alone		15	10	6		20.0
2347 L	AA117025	RT Alone		11.5	11.5	7		23.9
2348 R	AA117025	RT Alone		16	11	7		19.4
2349 LR	AA117025	RT Alone		12	11	5		21.7
2350	AA117026	RT Alone ϕ		16	10	6		21.3
2351	AA117026	RT Alone L		10	8	4		23.4
2352	AA117026	RT Alone R		10	10	4		21.7
2353	AA117026	RT Alone LR		13	9	1		22.1

Tuesday: Δ Cage for biohazard animals Done ✓

1863 ϕ	AA108971	10^7 3616+RT		4	3	2		24.1
1864 L	AA108971	10^7 3616+RT		11	10	3		25.5
1865 R	AA108971	10^7 3616+RT	<10 d38	ϕ	ϕ	ϕ		26.4
1866 LR	AA108971	10^7 3616+RT	<10 d31	ϕ	ϕ	ϕ		25.6
1883 ϕ	AA108966	10^7 3616+RT	<10 d24	ϕ	ϕ	ϕ		23.1
1884 L	AA108966	10^7 3616+RT	<10 d56	ϕ	ϕ	ϕ		23.4
1886 LR	AA108966	10^7 3616+RT	<10 d31	ϕ	ϕ	ϕ		19.8
2074 R	AA108966	10^7 3616+RT		11	10	3		14.7
2032 ϕ	AA111326	10^7 3616+RT		9	9	5		25.0
2033 L	AA111326	10^7 3616+RT		4	3	2		26.2
2035 LR	AA111326	10^7 3616+RT		10	7	3		25.1
2072 R	AA111326	10^7 3616+RT	<10 d21	ϕ	ϕ	ϕ		25.7
2036 ϕ	AA111327	10^7 3616+RT		7	5	3		24.8
2037 L	AA111327	10^7 3616+RT	<10 d52	2	2	2		27.2
2039 LR	AA111327	10^7 3616+RT		8	8	3		22.1
2073 R	AA111327	10^7 3616+RT		ϕ	ϕ	ϕ		21.7
1871 ϕ	AA108969	10^7 899-6+RT	<10 d66	ϕ	ϕ	ϕ		24.8
1873 R	AA108969	10^7 899-6+RT		5.5	5	2		25.1
1874 LR	AA108969	10^7 899-6+RT	<10 d52	ϕ	ϕ	ϕ		28.5
1880	AA108967	10^7 899-6+RT	<10 d14 L	ϕ	ϕ	ϕ		25.1
1881	AA108967	10^7 899-6+RT	<10 d42 R	8	9	3		27.5
1882	AA108967	10^7 899-6+RT	<10 d24 LR	ϕ	ϕ	ϕ		21.7
2044 ϕ	AA111329	10^7 899-6+RT		8	8	5		26.7
2046 R	AA111329	10^7 899-6+RT	<10 d77	ϕ	ϕ	ϕ		25.7
2048	AA111330	10^7 899-6+RT	<10 d38 ϕ	ϕ	ϕ	ϕ		13.5
2050	AA111330	10^7 899-6+RT	<10 d31 R	2	2	1		25.4
2051	AA111330	10^7 899-6+RT	LR	18	13	5		26.3

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control		15	16	14		
2354	AA117027	Control	sac	23	22	13		
2356	AA117027	Control		19.5	17	11		
2357	AA117027	Control		18	15	12		
2359	AA117028	Control	sac	23	20	15		
2360	AA117028	Control	sac	18	17	14.5		
2361	AA117028	Control	sac	22	23	16.5		
1710	AA105283	10^7 899-6	<10 d35	0				
2075	AA105283	10^7 899-6	<10 d42	0				
1713	AA105283	10^7 899-6	<10 d14	0				
2054	AA111331	10^7 899-6	<10 d49	0				
~~1723~~	~~AA104549~~	~~10^7 3616~~	~~<10 d28 face~~	0				
1859	AA108972	RT Alone	Sac'd ill	12	12	13		
1862	AA108972	RT Alone		4.5	6	4		
1892	AA108964	RT Alone		3.5	5	2.5		
2189	AA113941	RT Alone		+9.5	10	6.5		
2190	AA113941	RT Alone		5	4.5	2.5		
2192	AA113941	RT Alone		9.5	8	4		
2201	AA113944	RT Alone		8.5	7	3.5		
2202	AA113944	RT Alone		0				
2203	AA113944	RT Alone		10	9	5		
2204	AA113944	RT Alone		13	10.5	8.5		
2342	AA117024	RT Alone		12	10.5	6.5		
2343	AA117024	RT Alone		12	10	7		
2344	AA117024	RT Alone		15	12.5	8		
2345	AA117024	RT Alone		+10.5	8.5	5.5		
2346	AA117025	RT Alone		13	11	9		
2347	AA117025	RT Alone		12	10.5	9		
2348	AA117025	RT Alone		14.5	10	8		
2349	AA117025	RT Alone		11	9.5	5		
2350	AA117026	RT Alone		+14.5	10.5	7		
2351	AA117026	RT Alone		10	8	7		
2352	AA117026	RT Alone		9.5	8	6.5		
2353	AA117026	RT Alone		13.5	8.5	7		

17
7
119
x18
952
1190

16
7
112
x15
160
1120

1863	AA108971	10^7 3616+RT		5	4	2.5		
~~1864~~	~~AA108971~~	~~10^7 3616+RT~~		~~11.5~~	~~9.5~~	~~6.5~~		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	0				
1886	AA108966	10^7 3616+RT	<10 d31	0				
2074	AA108966	10^7 3616+RT		14	10	6		
2032	AA111326	10^7 3616+RT		8	9.5	8		
2033	AA111326	10^7 3616+RT		4	3.5	2		
2035	AA111326	10^7 3616+RT		9	8	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		6	5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		7	6	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	0				
1873	AA108969	10^7 899-6+RT		6	5	4		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	8.5	10	6.5		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		8.5	7.5	6		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
2048	AA111330	10^7 899-6+RT	<10 d38 sac	wt loss				
2050	AA111330	10^7 899-6+RT	<10 d31	0				
2051	AA111330	10^7 899-6+RT		21.5	16	10		

2209 AA113946 10^7 3616 8 6.5 7.5
10 impl 5/3 inj 6/8 7.5 6.5 5.5
11 10 7 6
12 8 8.5 6

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control		21	19	9		23.3
2354	AA117027	Control						
2356	AA117027	Control	dead					
2357	AA117027	Control	dead					
2359	AA117028	Control						
2360	AA117028	Control						
2361	AA117028	Control						
1710	AA105283	10^7 899-6	<10 d35	0				
2075	AA105283	10^7 899-6	<10 d42	0				
1713	AA105283	10^7 899-6	<10 d14	0				
2054	AA111331	10^7 899-6	<10 d49	0				
1723	AA104549	10^7 3616	<10 d28 face	0				
1859	AA108972	RT Alone						
1862	AA108972	RT Alone		5.5	6	4		25.1
1892	AA108964	RT Alone		5.5	3	2		25.3
2189	AA113941	RT Alone		9.5	11	7.5		24.8
2190	AA113941	RT Alone		3.5	3	2		20
2192	AA113941	RT Alone		8.5	8	4		24.2
2201	AA113944	RT Alone		6	7	4		22.5
2202	AA113944	RT Alone		0				27.8
2203	AA113944	RT Alone		9	10	4.5		26.5
2204	AA113944	RT Alone		14	11	8		25.5
2342	AA117024	RT Alone		14	15.5	6		23.6
2343	AA117024	RT Alone		10	9	6		23.4
2344	AA117024	RT Alone		12	10	6.5		24
2345	AA117024	RT Alone		10	12	4		23.8
2346	AA117025	RT Alone		13	9.5	7		19.4
2347	AA117025	RT Alone		12	11.5	8		23.4
2348	AA117025	RT Alone		15.5	9	8		18.4
2349	AA117025	RT Alone		13	10	7		20.7
2350	AA117026	RT Alone		14	9.5	7		21.8
2351	AA117026	RT Alone		12.5	8	6.5		22.9
2352	AA117026	RT Alone		9	8	5		23.4
2353	AA117026	RT Alone		10	8	5		24.3

1863	AA108971	10^7 3616+RT		5	4	3		23.8
1864	AA108971	10^7 3616+RT		9	8	4		26.3
1865	AA108971	10^7 3616+RT	<10 d38	0				25.8
1866	AA108971	10^7 3616+RT	<10 d31	0				25.7
1883	AA108966	10^7 3616+RT	<10 d24	0				22.8
1884	AA108966	10^7 3616+RT	<10 d56	1	1	1		21.5
1886	AA108966	10^7 3616+RT	<10 d31	0				19.6
2074	AA108966	10^7 3616+RT		19	10	7		15.8
2032	AA111326	10^7 3616+RT		8.5	8.5	7		24.3
2033	AA111326	10^7 3616+RT		3.5	3.5	2.5		26.6
2035	AA111326	10^7 3616+RT		8	9	4		24.9
2072	AA111326	10^7 3616+RT	<10 d21	0				25.9
2036	AA111327	10^7 3616+RT		4.5	5	3		22.8
2037	AA111327	10^7 3616+RT	<10 d52	0				25.9
2039	AA111327	10^7 3616+RT		7	4.5	2.5		21.2
2073	AA111327	10^7 3616+RT		0				21.1
1871	AA108969	10^7 899-6+RT	<10 d66	0				25.8
1873	AA108969	10^7 899-6+RT		4	7	3.5		26.1
1874	AA108969	10^7 899-6+RT	<10 d52	0				28.9
1880	AA108967	10^7 899-6+RT	<10 d14	0				20.4
1881	AA108967	10^7 899-6+RT	<10 d42	9	10.5	6		28.8
1882	AA108967	10^7 899-6+RT	<10 d24	0				24.3
2044	AA111329	10^7 899-6+RT		8	9.5	6		25.8
2046	AA111329	10^7 899-6+RT	<10 d77	0				25.4
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	4	2.5	1.5		26.2
2051	AA111330	10^7 899-6+RT	SCC	16	26	12		–

3616

2209	10	8.5	6	25.2
2210	9	7	5	25.2
2211	13	9.5	9.5	21.8
2212	8.5	9	6.5	22.6

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 24139

ARC USE ONLY

P.O. # ______

ORDER DATE ______

REF. # ______

CONTACT ______

EST AMT ______

SCHED DEL ______

SPECIAL ROUTING

NON COM VENDOR ______

FLAGGED BY PROTOCOL ______

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Greg Sibley, MD DATE: ______

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: ______

FAS ACCOUNT: 2-73731-5100

VENDOR: FCRI

REQUESTED DELIVERY DATE: ______

P.I.: Hallahan

PROTOCOL: 58671

PHONE: 2-0294

SPECIES: Mouse

QUANTITY: 80

STRAIN: Athymic Nude

SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE: ______

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

Deliver to Carlson J-013

Biohazard Suite

HOUSE AT: X CARLSON ___ WYLER ___ CLSC ___ FMI ___ OTHER

J-013

Greg's Mouse Log #2

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2354	DD117027	Control		None	None	
2355	DD117027	Control		None	None	
2356	DD117027	Control		None	None	
2357	DD117027	Control		None	None	
2358	DD117028	Control		None	None	
2359	DD117028	Control		None	None	
2360	DD117028	Control		None	None	
2361	DD117028	Control		None	None	
2667	AA12076	Control	18x16x13 22.6			
2668	AA12076	Control	17x13x10 22.6			
2669	AA12076	Control	16x15x11 22.3			
2670	AA12076	Control	20x14x10 22.6			

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif	
2663	AA120077	10^7 899-6			None		13x11x9 20.1
2664	AA120077	10^7 899-6			None		16x15x12 22.0
2665	AA120077	10^7 899-6			None		22.9
2666	AA120077	10^7 899-6			None		12x12x6 20.8
2703	AA120078	10^7 899-6					
2704		10^7 899-6					
2705		10^7 899-6					
2706		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif	
2659	AA120091	10^7 3616			None		21x15x11 21.9
2660	AA120091	10^7 3616			None		11x9x9 22.0
2661	AA120091	10^7 3616			None		15x11x9 22.0
2662	AA120091	10^7 3616			None		20x15x13 22.6
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					

Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif

2346	AA117025	RT Alone				
2347	AA117025	RT Alone				
2348	AA117025	RT Alone				
2349	AA117025	RT Alone				
2350	AA117026	RT Alone				
2351	AA117026	RT Alone				
2352	AA117026	RT Alone				
2353	AA117026	RT Alone				
2687	AA12093	RT Alone				
2688	AA12093	RT Alone				
2689	AA12093	RT Alone				
2690	AA12093	RT Alone				

Mouse #	Cage #	Group	Tumor Date	Inf. Date	RT Date	Date Sacrif
2679	AA120094	10^7 3616+RT				
2680	AA120094	10^7 3616+RT				
2681	AA120094	10^7 3616+RT				
2682	AA120094	10^7 3616+RT				
2691	AA120089	10^7 3616+RT				
2692	AA120089	10^7 3616+RT				
2693	AA120089	10^7 3616+RT				
2694	AA120089	10^7 3616+RT				
2683	AA120086	10^7 3616+RT				
2684	AA120086	10^7 3616+RT				
2685	AA120086	10^7 3616+RT				
2686	AA120086	10^7 3616+RT				

Mouse #	Cage #	Group	Tumor Date	Inf. Date	RT Date	Date Sacrif
2675	AA120080	10^7 899-6+RT				
2676	AA120080	10^7 899-6+RT				
2677	AA120080	10^7 899-6+RT				
2678	AA120080	10^7 899-6+RT				
2671	AA120085	10^7 899-6+RT				
2672	AA120085	10^7 899-6+RT				
2673	AA120085	10^7 899-6+RT				
2674	AA120085	10^7 899-6+RT				
2695	AA120088	10^7 899-6+RT				
2696	AA120088	10^7 899-6+RT				
2697	AA120088	10^7 899-6+RT				
2698	AA120088	10^7 899-6+RT				

2346	AA117025	RT Alone				
2347	AA117025	RT Alone				
2348	AA117025	RT Alone				
2349	AA117025	RT Alone				
2350	AA117026	RT Alone				
2351	AA117026	RT Alone				
2352	AA117026	RT Alone				
2353	AA117026	RT Alone				
2687	AA12093	RT Alone	11x9x8 20.1			
2688	AA12093	RT Alone	13x10x9 20.2			
2689	AA12093	RT Alone	13x10x9 21.4			
2690	AA12093	RT Alone	10x6x7 23.2			
Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2679	AA120084	10^7 3616+RT	7x6x4 17.5			
2680		10^7 3616+RT	10x7x4 14.2			
2681		10^7 3616+RT	13x10x8 21.4			
2682		10^7 3616+RT	12x9x9 16.4			
2691	AA120089	10^7 3616+RT	9x8x7 19.2			
2692		10^7 3616+RT	13x9x9 22.9			
2693		10^7 3616+RT	13x11x10 18.6			
2694		10^7 3616+RT	14x11x9 19.7			
2683	AA120086	10^7 3616+RT	9x7x6 19.0			
2684		10^7 3616+RT	7x6x4 15.0			
2685		10^7 3616+RT	7x6x4 19.7			
2686		10^7 3616+RT	11x7x7 15.0			
Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif
2675	AA120080	10^7 899-6+RT	10x9x8 21.1			
2676		10^7 899-6+RT	13x11x9 15.2			
2677		10^7 899-6+RT	14x11x9 16.6			
2678		10^7 899-6+RT	13x11x8 21.2			
2671	AA120085	10^7 899-6+RT	7x6x5 19.0			
2672		10^7 899-6+RT	11x8x6 20.8			
2673		10^7 899-6+RT	12x12x9 17.5			
2674		10^7 899-6+RT	17x13x9 15.9			
2695	AA120088	10^7 899-6+RT	9x9x7			
2696		10^7 899-6+RT	12x11x8			
2697		10^7 899-6+RT	9x9x8			
2698		10^7 899-6+RT	9x8x8			

Greg's Mouse Log #2

	Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif		
	2354	DD117027	Control		None	None			
	2355	DD117027	Control		None	None			
	2356	DD117027	Control		None	None			
	2357	DD117027	Control		None	None			
	2358	DD117028	Control		None	None			
	2359	DD117028	Control		None	None			
	2360	DD117028	Control		None	None			
	2361	DD117028	Control		None	None			
	2667	AA12076	Control 8x7x5.5		17x11x9	20.2			
	2668	AA12076	Control 9x6x5.5		14x10x11	21.1			
	2669	AA12076	Control 8x8x6		11x9x7	21.2			
	2670	AA12076	Control 6.5x9.5x5		13x10x9	21.6			
	Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif		
16x12x8	2663	AA120077	10^7 899-6			None		21x17x11	24.[illegible]
9x8x7	2664	AA120077	10^7 899-6			None		11x9x8	22.[illegible]
9x8x5	2665	AA120077	10^7 899-6			None		8x6x3	21
11x9x5	2666	AA120077	10^7 899-6			None		10x9x7	21.8
11x7x6	2703		10^7 899-6					[illegible]	
11x8x7	2704		10^7 899-6						
12x11x9	2705		10^7 899-6						
13x9x7	2706		10^7 899-6						
			10^7 899-6						
			10^7 899-6						
			10^7 899-6						
			10^7 899-6						
	Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif		
10x9x7	2659	AA120091	10^7 3616			None		8x7x5	21.[illegible]
11x9x6	2660	AA120091	10^7 3616			None		14x11x7	21.3
11x9x6	2661	AA120091	10^7 3616			None		11x8x5	22.4
10x7x4	2662	AA120091	10^7 3616			None		13x8x5	21.
			10^7 3616						
			10^7 3616						
			10^7 3616						
			10^7 3616						
			10^7 3616						
			10^7 3616						
			10^7 3616						
			10^7 3616						
	Mouse #	Cage #	Group	Tumor Date	Inf Date	RT Date	Date Sacrif		

Greg's Mouse Log #2

Mouse #	Cage #	Group		Tumor Date	Inj. Date	RT Date	Date Sacrif
2354	DD117027	Control			None	None	
2355	DD117027	Control			None	None	
2356	DD117027	Control			None	None	
2357	DD117027	Control			None	None	
2358	DD117028	Control			None	None	
2359	DD117028	Control			None	None	
2360	DD117028	Control			None	None	
2361	DD117028	Control			None	None	
2667	AA 12076	Control	8x7x5				
2668	AA 12076	Control	8x8x6				
2669	AA 12076	Control	8x7x4				
2670	AA 12076	Control	9x6x4				
2663	120097	10^7 899-6				None	
2664		10^7 899-6					
2665		10^7 899-6					
2666		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
		10^7 899-6					
2659	120091	10^7 3616				None	
2660		10^7 3616					
2661		10^7 3616					
2662		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					
		10^7 3616					

20 Gy
25 Gy day 2

Greg's Mouse Log

	Mouse #	Group					weight					weight	
	1710	10^7 899-6	0	0	0			0	0	0		26	
	2075	10^7 899-6	0	0	0			0	0	0		27	
	1713	10^7 899-6	0	0	0			0	0	0		25.9	
	2054	10^7 899-6	0	0	0			0	0	0		27.9	
	1723	10^7 3616	0	0	0			0	0	0		26.5	
	1862	RT Alone	11	7	4			11	8	6		26.5	
	1892	RT Alone	5	4	3			4	4	3		24	
	2189	RT Alone	10	10	7			11	10	8		25.5	
	2190	RT Alone	2.5	2	1.5			1.0	1.0	1.0		21.9	
	2192	RT Alone	6	6	1.5			6	5	1.5		24.5	
	2201	RT Alone	4	4	2.5			3	2	2		26.5	
	2202	RT Alone	0	0	0			0	0	0		[illegible]	29.5
	2203	RT Alone	9	8	5			9	7	7		24.1	
	2204	RT Alone	20	20	11			21	21	13		29.5	
	2342	RT Alone	18	17	9			18	17	10		28.8	
	2343	RT Alone	13	12	8			13	12	8		25	
	2344	RT Alone	14	12	7			12	12	9		24.7	
	2345	RT Alone	10	9	5			8	7	3		25.1	
	2346	RT Alone	11	12.5	9			12	10	7		23.1	
	2347	RT Alone	13.5	13.5	11.5			15	15	12		24.3	
	2348	RT Alone	11	16.5	12			13	18	11		20.1	
	2349	RT Alone	11	11	5.5			14	11	8		22.1	
	2350	RT Alone	13	9.75	8			14	10	8		24.6	
	2351	RT Alone	8.5	9.5	6			10	10	6		24.7	
	2352	RT Alone	9	9	6.25			10	8	6		24.1	
	2353	RT Alone	9	13	6			13	7	7		23.8	
	1863	10^7 3616+RT	3	3	3			4	3	2		25.3	
	1864	10^7 3616+RT	8	7	1.5			6	6	1.5		26.8	
	1865	10^7 3616+RT	0	0	0			0	0	0		27.1	
	1866	10^7 3616+RT	0	0	0			0	0	0		26.6	
	1883	10^7 3616+RT	0	0	0			0	0	0		24.2	
died 7	1884	10^7 3616+RT	0	0	0								
	1886	10^7 3616+RT	0	0	0			0	0	0		24.2	
	2032	10^7 3616+RT	12	10	7			11	9	7		25.8	
	2033	10^7 3616+RT	4	4	3			3	3	2.0		22.0	
	2035	10^7 3616+RT	6	6	5			6	5	3		24.8	
	2072	10^7 3616+RT	0	0	0			0	0	0		23.6	
	2036	10^7 3616+RT	5.5	5	2.5			[illegible]	[illegible]	2		[illegible]	23.?
	2037	10^7 3616+RT	0	0	0			0	0	0		21.1	
	2039	10^7 3616+RT	6.5	5	1.5			6	4	1.5		19.7	
	2073	10^7 3616+RT	0	0	0			0	0	0		21.9	
	1871	10^7 899-6+RT	0	0	0			0	0	0		25.8	
	1873	10^7 899-6+RT	0	0	0			0	0	0		26.1	
	1874	10^7 899-6+RT	0	0	0			0	0	0		21.2	
	1880	10^7 899-6+RT	0	0	0			0	0	0		24.5	
	1881	10^7 899-6+RT	15	12	8			16	13	10		19.7	
	1882	10^7 899-6+RT	0	0	0			0	0	0		19.7	
	2044	10^7 899-6+RT	15	12	7			14	12	7		27.0	
	2046	10^7 899-6+RT	0	0	0			0	0	0		26.9	
	2050	10^7 899-6+RT	2	2	1.5			0	0	0		22.4	

2346	AA117025	RT Alone	1		1	1	
2347	AA117025	RT Alone					
2348	AA117025	RT Alone					
2349	AA117025	RT Alone					
2350	AA117026	RT Alone					
2351	AA117026	RT Alone					
2352	AA117026	RT Alone					
2353	AA117026	RT Alone					
2687	AA11093	RT Alone	10x8x7		12x9x7		
2688		RT Alone	11x9x6		12x10x8		
2689		RT Alone	10x7x6		12x8x6		
2690		RT Alone	9x7x5		13x11x8		
2679		10^7 3616+RT	12 X8X 7				
2680		10^7 3616+RT				11x11x8	
2681		10^7 3616+RT				12x9x7	
2682		10^7 3616+RT				11x4x8	
2691		10^7 3616+RT				12x10x8	
2692		10^7 3616+RT				11x9x8	
2693		10^7 3616+RT				13x10x9	
2694		10^7 3616+RT				14x11x10	
2683		10^7 3616+RT				9x8x8	
2684		10^7 3616+RT				11x9x7	
2685		10^7 3616+RT				10x10x8	
2686		10^7 3616+RT				11x7x7	
2675		10^7 899-6+RT				12x11x8	
2676		10^7 899-6+RT				13x11x8	
2677		10^7 899-6+RT				11x10x9	
2678		10^7 899-6+RT				12x10x9	
2671		10^7 899-6+RT				11x7x7	
2672		10^7 899-6+RT				12x9x8	
2673		10^7 899-6+RT				12x10x9	
2674		10^7 899-6+RT				15x12x10	
		10^7 899-6+RT					
		10^7 899-6+RT					
		10^7 899-6+RT					
		10^7 899-6+RT					

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Greg's Mouse Log

Mouse #	Group					weight					weight
1710	10^7 899-6	Ø									
2075	10^7 899-6	Ø									
1713	10^7 899-6	Ø									
2054	10^7 899-6	Ø									
1723	10^7 3616	0	0	0							
1862	RT Alone	9	8	7							
1892	RT Alone	4	4	2							
2189	RT Alone	9	9	7							
2190	RT Alone	0	0	0							
2192	RT Alone	7	7	3							
2201	RT Alone	4	3	2							
2202	RT Alone	0	0	0							
2203	RT Alone	7	6	2							
Huge [illegible] 2204	RT Alone	22	22	17							
2342	RT Alone	17	17	10							
2343	RT Alone	11	10	7							
2344	RT Alone	13	11	7							
2345	RT Alone	7	5	1.5							
2346	RT Alone	12	9	7							
2347	RT Alone	17	14	9							
2348	RT Alone	14	12	10							
2349	RT Alone	13	10	7							
2350	RT Alone	14	10	7							
2351	RT Alone	10	8	7							
2352	RT Alone	9	8	6							
2353	RT Alone	11	9	7							
1863	10^7 3616+RT	3	2	2							
1864	10^7 3616+RT	Ø									
1865	10^7 3616+RT	Ø									
1866	10^7 3616+RT	Ø ~~[illegible]~~	~~[illegible]~~	~~[illegible]~~							
1883	10^7 3616+RT	0	0	0							
1884	10^7 3616+RT										
1886	10^7 3616+RT	0	0	0							
2032	10^7 3616+RT	12	10	8							
2033	10^7 3616+RT	2	2	1.5							
2035	10^7 3616+RT	8	5	2							
K 2072	10^7 3616+RT	0	0	0							
2036	10^7 3616+RT	5	4	2							
2037	10^7 3616+RT	0	0	0							
2039	10^7 3616+RT	2	2	1.5							
2073	10^7 3616+RT	0	0	0							
1871	10^7 899-6+RT	0	0	0							
1873	10^7 899-6+RT	0	0	0							
1874	10^7 899-6+RT	0	0	0							
1880	10^7 899-6+RT	0	0	0							
1881	10^7 899-6+RT	16	14	10							
1882	10^7 899-6+RT	0	0	0							
2044	10^7 899-6+RT	14	12	10							
2046	10^7 899-6+RT	0	0	0							
2050	10^7 899-6+RT	2.5	2	1.5							

Greg's Mouse Log

Mouse #	Group					weight					weight
1710	10^7 899-6	0	-	-		26.9					
2075	10^7 899-6	0	0	0		26.7					
1713	10^7 899-6	0	-	-		23.0					
2054	10^7 899-6	0	-	-		25.1					
1723	10^7 3616	0	-	-	27.8	24.2					
1862	RT Alone	11	8	7		26.9					
1892	RT Alone	3	3	2		26.1					
2189	RT Alone	0	7	5		26 25.2					
2190	RT Alone	0				25.8					
2192	RT Alone	4	4	1.5		25.0					
2201	RT Alone	3	3	1.5		27.3					
2202	RT Alone	0	-	-		24.6					
2203	RT Alone	5	4	1.5		25.6					
2204	RT Alone	Sac'd 4/22									
2342	RT Alone	17	17	12		25.0					
2343	RT Alone	12	11	8		24.3					
2344	RT Alone	12	9	8		24.7					
2345	RT Alone	7	3	1.5		25.5					
2346	RT Alone	12	10	7		23.4					
2347	RT Alone	18	15	11		24.3					
2348	RT Alone	15	13	9		20.4					
2349	RT Alone	14	10	8		21.2					
2350	RT Alone	13	10	8		25.4					
2351	RT Alone	8	7	6		25.1					
2352	RT Alone	9	8	5		24.6					
2353	RT Alone	11	7	4		25.5					
1863	10^7 3616+RT	3	3	1		24.7					
1864	10^7 3616+RT	0	-	-		27.7					
1865	10^7 3616+RT	0	-	-		27.3					
1866	10^7 3616+RT	0	-	-		26.4					
1883	10^7 3616+RT	0	-	-		24.5					
1884	10^7 3616+RT										
1886	10^7 3616+RT	0	-	-		25.4					
2032	10^7 3616+RT	12	9	8		25.7					
2033	10^7 3616+RT	2	2	1		26.6					
2035	10^7 3616+RT	3	2	1		24.7					
2072	10^7 3616+RT	0	-	-		23.9					
2036	10^7 3616+RT	3	3	2		21.4					
2037	10^7 3616+RT	1	1	1		26.4					
2039	10^7 3616+RT	0	-	-		19.0					
2073	10^7 3616+RT	0	-	-		21.3					
1871	10^7 899-6+RT	0	-	-		26.4					
1873	10^7 899-6+RT	0	-	-		27.2					
1874	10^7 899-6+RT	0	-	-		25.6					
1880	10^7 899-6+RT	0	-	-		19.8					
1881	10^7 899-6+RT	15	13	11		27.2					
1882	10^7 899-6+RT	0	-	-		24.0					
2044	10^7 899-6+RT	11	10	7	26.4 →						
2046	10^7 899-6+RT	0	-	-	27.0 →						
2050	10^7 899-6+RT	1	1	1	27.0 →						

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control						
2354	AA117027	Control						
2356	AA117027	Control						
2357	AA117027	Control						
2359	AA117028	Control						
2360	AA117028	Control						
2361	AA117028	Control						
1710	AA105283	10^7 899-6	<10 d35	0				
2075	AA105283	10^7 899-6	<10 d42	0				
1713	AA105283	10^7 899-6	<10 d14	0				
2054	AA111331	10^7 899-6	<10 d49	0				
1723	AA104549	10^7 3616	<10 d28 face	0				
~~1859~~	AA108972	RT Alone						
1862	AA108972	RT Alone		5	6	4.5		
1892	AA108964	RT Alone		4.5	3	2.5		
2189	AA113941	RT Alone		10	13	6		
2190	AA113941	RT Alone		4	3	1.5		
2192	AA113941	RT Alone		8	7	4		
2201	AA113944	RT Alone		6	7	4		
2202	AA113944	RT Alone		0				
2203	AA113944	RT Alone		9	9	4		
2204	AA113944	RT Alone		13.5	11	9		
2342	AA117024	RT Alone		8	13.5	5.5		
2343	AA117024	RT Alone		10	9	6		
2344	AA117024	RT Alone		11.5	9	6.5		
2345	AA117024	RT Alone		13	14.5	9		
2346	AA117025	RT Alone		13	11	8		
2347	AA117025	RT Alone		10.5	10.5	7		
2348	AA117025	RT Alone		15	9.5	8.5		
2349	AA117025	RT Alone		10	14	7.5		
2350	AA117026	RT Alone		14	10.5	7		
2351	AA117026	RT Alone		10	8	6		
2352	AA117026	RT Alone		9.5	8.5	6		
2353	AA117026	RT Alone		12	9	~~6~~ 7		

3616

2209

2210

2211

2212

1863	AA108971	10^7 3616+RT		4.5	3	2.5		
1864	AA108971	10^7 3616+RT		8.5	6	3		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	1.5	1.5	1.5		
1886	AA108966	10^7 3616+RT	<10 d31	0				
~~2074~~	AA108966	10^7 3616+RT	sac wt loss	21.4	13.5	7		
2032	AA111326	10^7 3616+RT		8	8	6		
2033	AA111326	10^7 3616+RT		3	2	1.5		
2035	AA111326	10^7 3616+RT		8.5	8.5	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		5	4.5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		6	4.5	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	6	4.5	3		
1873	AA108969	10^7 899-6+RT		4.5	5.5	3.5		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	10	10.5	6		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		9	8	9		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
~~2048~~	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	0				
~~2051~~	AA111330	10^7 899-6+RT						

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control						
2354	AA117027	Control						
2356	AA117027	Control						
2357	AA117027	Control						
2359	AA117028	Control						
2360	AA117028	Control						
2361	AA117028	Control						
1710	AA105283	10^7 899-6	<10 d35					24.9
2075	AA105283	10^7 899-6	<10 d42					24.8
1713	AA105283	10^7 899-6	<10 d14					22.4
2054	AA111331	10^7 899-6	<10 d49					23.7
1723	AA104549	10^7 3616	<10 d28 face	0				26.7
~~1859~~	AA108972	RT Alone						
1862	AA108972	RT Alone		5	7	4.5		26.3
1892	AA108964	RT Alone		3.5	3.5	1.5		25.7
2189	AA113941	RT Alone		10	13	9		25.7
2190	AA113941	RT Alone		4	3	2		21.7
2192	AA113941	RT Alone		8	7.5	2.5		24.7
2201	AA113944	RT Alone		6	6	3		20.4
2202	AA113944	RT Alone		0				26.2
2203	AA113944	RT Alone		9.5	9	4		23.1
2204	AA113944	RT Alone		16	12	8		26.2
2342	AA117024	RT Alone		13.5	13.5	8		24
2343	AA117024	RT Alone		11	9	7		24.3
2344	AA117024	RT Alone		11.5	10	5		24.0
2345	AA117024	RT Alone		9.5	7	3		24.9
2346	AA117025	RT Alone		12	11	8		21.0
2347	AA117025	RT Alone		12	11.5	7		23.8
2348	AA117025	RT Alone		14	9.5	9		18.8
2349	AA117025	RT Alone		11	12	6.5		20.3
2350	AA117026	RT Alone		10	8	5.5		24.0
2351	AA117026	RT Alone		9.5	15	7		23.8
2352	AA117026	RT Alone		9	8.5	5		23.5
2353	AA117026	RT Alone		12.5	8	7		22

1863	AA108971	10^7 3616+RT		4	3	2		24.9
1864	AA108971	10^7 3616+RT		7	4.5	2.5		27.0
1865	AA108971	10^7 3616+RT	<10 d38	0				27.1
1866	AA108971	10^7 3616+RT	<10 d31	0				26.3
1883	AA108966	10^7 3616+RT	<10 d24	0				22.6
1884	AA108966	10^7 3616+RT	<10 d56	0				21.2
1886	AA108966	10^7 3616+RT	<10 d31	0				20.8
2074	AA108966	10^7 3616+RT						
2032	AA111326	10^7 3616+RT		7.5	10	7		25.7
2033	AA111326	10^7 3616+RT		3.5	4.5	2.5		26.3
2035	AA111326	10^7 3616+RT		7	7.5	4		24.7
2072	AA111326	10^7 3616+RT	<10 d21	6.5	5.5	3		26.0
2036	AA111327	10^7 3616+RT		5	4.5	3		23.2
2037	AA111327	10^7 3616+RT	<10 d52	0				27
2039	AA111327	10^7 3616+RT		5.5	4	2.5		20.8
2073	AA111327	10^7 3616+RT		0				22
1871	AA108969	10^7 899-6+RT	<10 d66	5	5	3		26.5
1873	AA108969	10^7 899-6+RT		6	5	3		26.3
1874	AA108969	10^7 899-6+RT	<10 d52	0				28.1
1880	AA108967	10^7 899-6+RT	<10 d14	0				19.8
1881	AA108967	10^7 899-6+RT	<10 d42	9.5	13	8		30
1882	AA108967	10^7 899-6+RT	<10 d24	0				24
2044	AA111329	10^7 899-6+RT		12.5	10	9.5		27.4
2046	AA111329	10^7 899-6+RT	<10 d77	0				25.4
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	2.5	2.5	1		26.4
2051	AA111330	10^7 899-6+RT						

Greg's Mouse Log

Mouse #	Group					weight					weight
1710	10^7 899-6										
2075	10^7 899-6										
1713	10^7 899-6										
2054	10^7 899-6										
1723	10^7 3616	0									
1862	RT Alone	8	6	5			8	6	5		
1892	RT Alone	3	3	2			3	3	2		
2189	RT Alone	14	10	9			11	12.5	8		
2190	RT Alone	2	2	1			0				
2192	RT Alone	6	5	3			6	7.5	2		
2201	RT Alone	0					5.5	5	3		
2202	RT Alone	8	8	5			0				
2203	RT Alone	6	6	4			8	8.5	4.5		
2204	RT Alone	17	13	11			17.5	16	14		
2342	RT Alone						16.9	14	9		
2343	RT Alone						10.5	10	6		
2344	RT Alone						11	11	7		
2345	RT Alone						14	7.5	4		
2346	RT Alone	13	12	9			13	11	8		
2347	RT Alone	15	14	7			12.5	14	6.5		
2348	RT Alone	16	11	7			15	9.5	7		
2349	RT Alone	12	10	8			10	15	6		
2350	RT Alone	12	8	4			10	14.5	8		
2351	RT Alone	7	3	3			9	7.5	6		
2352	RT Alone	5	5	2			9	8	5		
2353	RT Alone	9	6	6			12.5	9	6.5		
1863	10^7 3616+RT	9	4	2							
1864	10^7 3616+RT	8	7	2							
1865	10^7 3616+RT	0									
1866	10^7 3616+RT	0									
1883	10^7 3616+RT	0									
1884	10^7 3616+RT	0									
1886	10^7 3616+RT	0									
2032	10^7 3616+RT										
2033	10^7 3616+RT										
2035	10^7 3616+RT										
2072	10^7 3616+RT										
2036	10^7 3616+RT										
2037	10^7 3616+RT										
2039	10^7 3616+RT										
2073	10^7 3616+RT										
1871	10^7 899-6+RT										
1873	10^7 899-6+RT										
1874	10^7 899-6+RT										
1880	10^7 899-6+RT										
1881	10^7 899-6+RT										
1882	10^7 899-6+RT										
2044	10^7 899-6+RT										
2046	10^7 899-6+RT										
2050	10^7 899-6+RT										

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Greg's Mouse Log

Mouse #	Group					weight					weight
1710	10^7 899-6	0									
2075	10^7 899-6	0									
1713	10^7 899-6	0									
2054	10^7 899-6	0									
1723	10^7 3616										
1862	RT Alone										
1892	RT Alone										
2189	RT Alone										
2190	RT Alone										
2192	RT Alone										
2201	RT Alone										
2202	RT Alone										
2203	RT Alone										
2204	RT Alone										
2342	RT Alone	16.5	16	7							
2343	RT Alone	11.5	8	5							
2344	RT Alone	11.5	11	6							
2345	RT Alone	10	7	4							
2346	RT Alone										
2347	RT Alone										
2348	RT Alone										
2349	RT Alone										
2350	RT Alone										
2351	RT Alone										
2352	RT Alone										
2353	RT Alone										
1863	10^7 3616+RT										
1864	10^7 3616+RT										
1865	10^7 3616+RT										
1866	10^7 3616+RT										
1883	10^7 3616+RT										
1884	10^7 3616+RT										
1886	10^7 3616+RT										
2032	10^7 3616+RT	10	11	5							
2033	10^7 3616+RT	3	3	2							
2035	10^7 3616+RT	8	7	3.5							
2072	10^7 3616+RT	0									
2036	10^7 3616+RT	5.5	4	3							
2037	10^7 3616+RT	0									
2039	10^7 3616+RT	4.5	3	2							
2073	10^7 3616+RT	0									
1871	10^7 899-6+RT	0									
1873	10^7 899-6+RT	7	6	3.5							
1874	10^7 899-6+RT	0									
1880	10^7 899-6+RT	0									
1881	10^7 899-6+RT	0									
1882	10^7 899-6+RT	11.5	14	6							
2044	10^7 899-6+RT	14	12	11							
2046	10^7 899-6+RT	0									
2050	10^7 899-6+RT	0									

Greg's Mouse Log

Mouse #	Group				weight					weight
1710	10^7 899-6	0			25.8					
2075	10^7 899-6	0			25.2					
1713	10^7 899-6	0			22.7					
2054	10^7 899-6	0			24.3					
1723	10^7 3616	0			26.3					
1862	RT Alone	8	7	4	23.2					
1892	RT Alone	4	4	2	22.9					
2189	RT Alone	14	11	10	25.3					
2190	RT Alone	0			22.0					
2192	RT Alone	6	6	6	25.0					
2201	RT Alone	5	5	4	28.0					
2202	RT Alone	0			27.4					
2203	RT Alone	9	9	6	23.3					
2204	RT Alone	18	17	10	27.6					
2342	RT Alone	13	12	6	25.0					
2343	RT Alone	12	7	7	26.3					
2344	RT Alone	19	15	9	25.3					
2345	RT Alone	10	8	5	25.7					
2346	RT Alone	11	11	8	22.8					
2347	RT Alone	15	14	9	26.0					
2348	RT Alone	16	9	7	18.3					
2349	RT Alone	16	16.5	6	22.2					
2350	RT Alone	15	11	6	25.5					
2351	RT Alone	9	8	5	24.2					
2352	RT Alone	9	9	4	24.9					
2353	RT Alone	11	12	6	23.3					
1863	10^7 3616+RT	4	4	2	25.3					
1864	10^7 3616+RT	9	8	1.5	25.6					
1865	10^7 3616+RT	0			27.4					
1866	10^7 3616+RT	0			26.7					
1883	10^7 3616+RT	0			23.4					
1884	10^7 3616+RT	0			19.5					
1886	10^7 3616+RT	0			22.6					
2032	10^7 3616+RT	0			26.0					
2033	10^7 3616+RT	4	3	1.5	26.5					
2035	10^7 3616+RT	7	6	4	24.7					
2072	10^7 3616+RT	9	7	5	26.8					
2036	10^7 3616+RT	6	5	1.5	23.9					
2037	10^7 3616+RT	0			27.3					
2039	10^7 3616+RT	5	5	1.5	19.9					
2073	10^7 3616+RT	0			22.5					
1871	10^7 899-6+RT	0			25.9					
1873	10^7 899-6+RT	7	6	1.5	26.1					
1874	10^7 899-6+RT	0			27.0					
1880	10^7 899-6+RT	0			19.8					
1881	10^7 899-6+RT	15	12	7	30.1					
1882	10^7 899-6+RT	0			23.9					
2044	10^7 899-6+RT	14	14	7	28.2					
2046	10^7 899-6+RT	0			26.3					
2050	10^7 899-6+RT	0			26.4					

Data Sheet

	2679	10^7 3636RT	6x6x3	5x5x2	
	2680	10^7 3616RT	8x6x3	7x4x2	
	2681	10^7 3636RT	12x10x7	11x9x4	
	2682	10^7 3616RT	13x8x8	13x8x6	
	2691	10^7 3636RT	~~13x10x9~~ [illegible]	[illegible]	21.5
	2692	10^7 3616RT	11x8x8	14x10x8	23.2
	2693	10^7 3636RT	13x10x8	[illegible]x6	22.6
	2694	10^7 3616RT	12x9x9	11x9x6	25.7
	2683	10^7 3616RT	8x7x6	8x7x4	21.4
	2684	10^7 3636RT	7x7x5	6x5x3	17.1
	2685	10^7 3616RT	8x7x5	6x4x3	23.1
	2686	10^7 3616RT	10x7x7	8x6x6	A3
	2675	10^7 8996RT	10x9x7	[illegible]	10x7x5
	2676	10^7 8996RT	12x12x9	13x9x8	
	2677	10^7 8996RT	14x9x8	12x9x4	
	2678	10^7 8996RT	14x9x9	13x10x6	
	2671	10^7 8996RT	8x6x4	6x5x4	
	2672	10^7 8996RT	9x7x6	8x6x5	
	2673	10^7 8996RT	10x10x9	13x11x6	
	2674	10^7 8996RT	10x8x4	9x8x1.5	
	2695	10^7 8996RT		10x8x8	
	2696	10^7 8996RT		17x12x9	
INJ	2697	10^7 8996RT		10x10x7	
	2698	10^7 8996RT		11x11x10	
	2663	899-6	13x10x9	16x12x8	
	2664	899-6	23x13x15	27x25x15	⇒ SAC
	2665	899-6	~~sac 7/6~~		
	2666	899-6	14x9x8	19x15x10	
	2703	899-6	9x7x5	[illegible]	11x7x4
	2704	899-6	11x9x6	11x11x3	
	2705	899-6	14x12x10	18x14x7	
	2706	899-6	16x11x9	17x11x9	
		899-6			
		899-6			
		899-6			
		899-6			

Data Sheet

Greg's Mouse	Data				
Mouse#	Group	Measurements	Weight		
2189	RT Alone	6x6x3	7x6x3	26.7	
2190	RT Alone	0x--	0	23.4	
2192	RT Alone	2x2x1	1x1x1	25.9	
2201	RT Alone	2x2x1.5	3x3x1.5	29.2	
2202	RT Alone	0x--	0	29.9	
2203	RT Alone	4x4x3	4x3x3	23.5	
~~[illegible]~~	~~RT Alone~~		SAC		
2342	RT Alone	11x10x9	11x9x3	21.3	
2343	RT Alone	12x10x7	~~[illegible]~~	24.4	11x10x7
2344	RT Alone	13x12x13	19x17x12	24.0	
2345	RT Alone	7x3x1.5	6x3x1.5	24.5	
2346	RT Alone	12x10x7	9x7x5	23.4	
2347	RT Alone	~~13x12x7~~ 13x15x12	17x15x11	26.3	
2348	RT Alone	13x12x8	16x14x9	21.4	
2349	RT Alone	14x10x10	14x10x8	17.4	
sick 2350	RT Alone	12x9x8	12x9x6	26.1	
2351	RT Alone	8x6x5	9x7x4	21.9	
2352	RT Alone	9x8x4	8x8x3	26.0	
2353	RT Alone	11x6x5	9x6x4	25.1	
2687	RT Alone	12x10x8	11x9x7	21.0	
2688	RT Alone	13x11x9	~~[illegible]~~	[illegible]	12x11x8 21.8
2689	RT Alone	13x8x8	14x8x7	23.9	
2690	RT Alone	10x7x5	8x7x6	24.5	
2354	Control				
2355	Control				
2356	Control				
2357	Control				
2358	Control				
2359	Control				
2360	Control				
2361	Control				
* 2667	Control	19x16x15		deceased	
2668	Control	16x15x12	16x14x13	18.5	
2669	Control	16x15x11	18x16x13	23.0	
2670	Control	22x15x12	22x15x10		

Data Sheet

	2659	3616	14 x 14 x 14	15 x 15 x 15	
	2660	3616	22 x 18 x 13	28 x 19 x 11	⇒ SAC
	2661	3616	19 x 15 x 11	18 x 16 x 7	
[illegible]	2662	3616	23 x 15 x 15	27 x 17 x 12	⇒ SAC
AA12008?	2707	3616	13 x 10 x 7		[illegible]
	2708	3616	12 x 9 x 6		
	2709	3616	11 x 9 x 7		
	2710	3616	13 x 11 x 8		
AA 12081	2719	3616	9 x 8 x 6		
	2720	3616	7 x 8 x 7		
	2721	3616	9 x 8 x 7		
	2722	3616	10 x 8 x 6		

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2189	RT Alone	9x5x1.5	25.8
2190	RT Alone	0	23.9
2192	RT Alone	3x3x2	25.9
2201	RT Alone	5x2x1.5	28.1
2202	RT Alone	0	29.3
2203	RT Alone	4x3x1.5	24.2
2204 SAC	RT Alone		
2342	RT Alone	11x9x4	21.9
2343	RT Alone	12x12x6	21.1
2344	RT Alone	22x14x10	24.0
2345	RT Alone	6x3x1.5	24.3
2346	RT Alone	9x8x5	23.5
2347	RT Alone	16x16x10	25.2
2348	RT Alone	15x15x10	21.0
~~2349~~	~~RT Alone~~	died 7/20/15	
2350	RT Alone	8x8x3	24.3
2351	RT Alone	12x9x5	27.1
2352	RT Alone	8x8x3	25.7
2353	RT Alone	9x6x4	25.3
2687	RT Alone	12x9x6	22.0
2688	RT Alone	12x10x6	23.5
2689	RT Alone	12x8x5	24.7
2690	RT Alone	9x7x6	25.1
2354	Control		
2355	Control		
2356	Control		
2357	Control		
2358	Control		
2359	Control		
2360	Control		
2361	Control		
2667 SAC	Control		
2668	Control		
2669	Control	19x14x14	22.7
2670	Control	21x13x8	17.4

Data Sheet

		[illegible]	[illegible]
2679	10^7 3636RT	5x4x1.5	22.4
2680	10^7 3616RT	7x4x1.5	13.7
2681	10^7 3636RT	11x9x5	24.2
2682	10^7 3616RT	13x8x8	20.6
2691	10^7 3636RT	7x6x4	23.5
2692	10^7 3616RT	9x8x6	27.1
2693	10^7 3636RT	12x10x3	22.6
2694	10^7 3616RT	13x9x7	24.1
2683	10^7 3616RT	7x6x4	28.2
2684	10^7 3636RT	0	19.0
2685	10^7 3616RT	6x5x1.5	23.4
2686	10^7 3616RT	10x8x4	18.9
2675	10^7 8996RT	12x8x5	25.2
2676	10^7 8996RT	13x12x7	17.3
2677	10^7 8996RT	12x9x5	19.1
2678	10^7 8996RT	12x10x6	25.8
2671	10^7 8996RT	7x7x4	22.0
2672	10^7 8996RT	8x6x4	25.1
2673	10^7 8996RT	14x11x6	20.1
2674	10^7 8996RT	11x8x4	14.4
2695	10^7 8996RT	10x9x6	22.7
2696	10^7 8996RT	17x13x9	19.7
2697	10^7 8996RT	10x10x6	24.9
2698	10^7 8996RT	12x10x8	17.1
2663	899-6	16x15x7	22.4
2664 SAC	899-6		
2665	899-6	21x17x13	24.3
SAC ~~2666~~	899-6		
2703	899-6	13x8x5	25.2
2704	899-6	12x11x4	27.3
2705	899-6	21x15x9	26.3
2706	899-6	19x13x6	21.0
	899-6		
	899-6		
	899-6		
	899-6		

		" "	
2659	3616	20 x 20 x 14	24.8
2660 SAC	3616		
2661	3616	21 x 16 x 12	23.2
2662 SAC	3616		
2707	3616		
2708	3616		
2709	3616		
2710	3616		
2719	3616		
2720	3616		
2721	3616		
2722	3616		

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2189	RT Alone	8x8x1.5	5x5x1.5	28.3
2190	RT Alone	Ø	Ø	25.9
2192	RT Alone	Ø	Ø	23.3
2201	RT Alone	5x3x1.5	5x3x1.5	29.8
2202	RT Alone	Ø	Ø	29.1
2203	RT Alone	5x4x1.5	6x4x1.5	23.3
~~2204 SAC~~	~~RT Alone~~			
★ 2342	RT Alone	20x17x12	22x17x12	24.6
2343	RT Alone	12x10x5	12x10x6	24.7
2344	RT Alone	Ø	Ø	23.1
2345	RT Alone	Ø	Ø	24.7
2346	RT Alone	11x7x3	8x7x3	25.1
2347	RT Alone	15x15x10	17x15x10	26.0
2348	RT Alone	15x15x9	14x11x4	20.8
SAC ~~2349~~	~~RT Alone~~			
2350	RT Alone	12x9x6	10x9x5	27.2
2351	RT Alone	10x8x4	10x7x3	25.5
2352	RT Alone	9x9x1.5	9x9x1.5	26.2
2353	RT Alone	10x7x2	9x6x4	25.7
2687	RT Alone	11x9x6	11x10x4	28.6
2688	RT Alone	12x9x5	12x9x4	26.3
2689	RT Alone	11x9x8	12x9x4	24.3
2690	RT Alone	10x8x4	9x7x4	27.3
~~2354~~	~~Control~~			
~~2355~~	~~Control~~			
~~2356~~	~~Control~~			
~~2357~~	~~Control~~			
~~2358~~	~~Control~~			
~~2359~~	~~Control~~			
~~2360~~	~~Control~~			
~~2361~~	~~Control~~			
~~2667 SAC~~	~~Control~~			
~~2668~~	~~Control~~			
~~2669~~	~~Control~~			
~~2670~~	~~Control~~			

Data Sheet

2679	10^7 3636RT	Ø	Ø	23.8
2680	10^7 3616RT	4x2x1.5	Ø	13.0
2681	10^7 3636RT	10x9x3	10x8x3	23.7
2682	10^7 3616RT	13x9x7	12x8x6	20.9
2691	10^7 3636RT	~~[illegible]~~ 6x5x2	6x4x2	24.3
2692	10^7 3616RT	11x8x5	11x9x5	25.5
2693	10^7 3636RT	11x11x4	10x8x4	29.0
2694	10^7 3616RT	13x10x6	10x9x3	18.6
2683	10^7 3616RT	9x7x2	6x5x1.5	23.6
2684	10^7 3636RT	Ø	Ø	24.2
2685	10^7 3616RT	3x1.5x1.5	Ø	23.2
2686	10^7 3616RT	7x6x4	7x5x3	21.4
2675	10^7 8996RT	7x7x4	11x11x5	19.6
2676	10^7 8996RT	12x11x6	7x5x2	27.4
2677	10^7 8996RT	12x9x3	10x7x3	28.2
2678	10^7 8996RT	10x10x4	9x9x4	28.4
2671	10^7 8996RT	5x4x4	5x4x2	23.0
2672	10^7 8996RT	5x3x1.5	5x3x1.5	26.4
2673	10^7 8996RT	11x9x7	9x7x4	20.3
2674	10^7 8996RT	8x6x2	6x5x1.5	17.4
2695	10^7 8996RT	10x9x5	8x5x3	21.6
2696	10^7 8996RT	10x9x6	12x9x5	17.3
2697	10^7 8996RT	13x12x6	8x7x5	26.6
2698	10^7 8996RT	12x9x6	16x13x7	21.1
2663	899-6	15x12x6	16x10x4	20.4
~~2664 SAC~~	~~899-6~~			
★ SAC 2665	899-6	23x19x14	←	SAC
SAC ~~2666~~	~~899-6~~			
2703	899-6	17x12x4	17x14x8	26.2
2704	899-6	15x13x6	19x15x6	29.6
2705	899-6	19x17x9	19x15x10	21.0
2706	899-6	20x15x8	21x15x8	27.4
— [illegible]	899-6			
— [illegible]	899-6	[illegible]		
	899-6			
	899-6			

~~SAC 2659~~	~~3616~~	21x21x15		
~~2660 SAC~~	~~3616~~			
~~SAC 2661~~	~~3616~~	25x19x13		
~~2662 SAC~~	~~3616~~			
2707	3616	11x9x7	11x9x4	23.8
2708	3616	12x10x6	12x10x5	22.8
2709	3616	14x12x7	14x13x10	25.0
2710	3616	13x12x7	14x11x8	25.0
2719	3616	7x7x4	6x5x15	22.0
2720	3616	11x9x5	8x6x3	25.1
2721	3616	7x6x4	13x9x6	23.3
2722	3616	9x8x4	9x6x4	22.7

Data Sheet

	Greg's Mouse	Data			
	Mouse#	Group	Measurements	Weight	
	2189	RT Alone	Ø	Ø	23.5
	2190	RT Alone	Ø	Ø	22.4
	2192	RT Alone	Ø	Ø	25.0
	2201	RT Alone	Ø	Ø	28.0
	2202	RT Alone	5x3x1.5	Ø	28.4
	2203	RT Alone	4x4x1.5	4x4x1.5	22.9
	SAC ~~2204~~	~~RT Alone~~			
	2342	RT Alone	23x19x7	23x19x9	24.0
	2343	RT Alone	11x10x6	12x11x5	23.5
	2344	RT Alone	Ø	Ø	23.0
	2345	RT Alone	Ø	Ø	23.5
	2346	RT Alone	Ø	Ø	21.3
	2347	RT Alone	19x17x11	19x17x11	26.8
	2348	RT Alone	14x12x10	14x12x10	21.6
	SAC ~~2349~~	~~RT Alone~~			
	2350	RT Alone	9x8x3	9x8x3	24.4
	2351	RT Alone	8x7x3	9x6x2	26.4
	2352	RT Alone	10x9x3	8x8x1.5	26.6
	2353	RT Alone	9x5x1.5	8x5x2	25.5
	2687	RT Alone	10x7x4	11x7x5	24.0
	2688	RT Alone	9x9x4	11x11x4	25.6
	2689	RT Alone	14x13x6	15x13x9	24.1
	2690	RT Alone	8x7x2	9x7x3	26.9
	2679	10^7 3636RT	Ø	Ø	22.7
died	~~2680~~	~~10^7 3616RT~~	Ø		
	2681	10^7 3636RT	9x7x3	8x6x2	15.6
	2682	10^7 3616RT	12x6x4	12x6x5	21.5
	2691	10^7 3636RT	4x4x1.5	3x3x1.5	23.2
	2692	10^7 3616RT	10x6x3	8x6x2	27.7
	2693	10^7 3636RT	8x7x3	8x7x1.5	23.4
	2694	10^7 3616RT	11x9x4	11x9x4	25.9
	2683	10^7 3616RT	6x4x1.5	3x3x1.5	23.3
	2684	10^7 3636RT	Ø	Ø	21.8
	2685	10^7 3616RT	Ø	Ø	25.1
	2686	10^7 3616RT	5x5x2	4x4x1.5	20.8
	2675	10^7 8996RT	7x5x1.5	6x5x2	27.1
	2676	10^7 8996RT	10x9x4.5	8x8x4	19.1
	2677	10^7 8996RT	7x6x2	5x4x1.5	22.6
	2678	10^7 8996RT	8x8x3	8x8x3	28.0

Data Sheet

	2671	10^7 8996RT	2x2x1.5	2x2x1.5	22.2
	2672	10^7 8996RT	5x3x1.5	2x2x1.5	25.5
	2673	10^7 8996RT	9x7x3	9x7x2	18.6
	2674	10^7 8996RT	6x4x1.5	4x3x1.5	22.9
	2695	10^7 8996RT	7x6x3	6x5x2	21.7
	2696	10^7 8996RT	~~[illegible]~~ [illegible]	16x11x7	20.9
	2697	10^7 8996RT	9x7x6	9x7x3	27.5
	2698	10^7 8996RT	10x9x5	11x9x4	16.8
	2663	899-6	16x11x5	17x13x6	21.2
	~~SAC 2664~~	~~899-6~~			
	~~SAC 2665~~	~~899-6~~			
	~~SAC 2666~~	~~899-6~~			
	2703	899-6	21x16x7	21x19x7	27.3
	2704	899-6	22x16x6	23x15x5	21.5
die	2705	899-6	14x15x8		
	2706	899-6	18x18x6	17x15x4	19.7
injected	2711	899-6	15x13x8		
	2712	899-6	13x10x8		
	2713	899-6	11x11x10		
	2714	899-6	14x10x10		
	2707	3616	13x10x4	15x13x6	27.2
	2708	3616	11x10x6	12x11x6	23.4
	2709	3616	~~[illegible]~~ [illegible]	20x16x9	26.9
	2710	3616	17x13x7	17x15x10	25.7
	2719	3616	5x3x1.5	2x2x1.5	23.9
	2720	3616	5x4x1.5	2x2x1.5	26.3
	2721	3616	11x9x3	13x11x6	24.9
	2722	3616	7x6x2	6x6x4	24.5

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2189	RT Alone	1.5x1.5x1	1.0x1.0x1	25.0
2190	RT Alone	Ø	Ø	24.3
2192	RT Alone	Ø	Ø	26.3
2201	RT Alone	Ø	Ø	29.7
2202	RT Alone	Ø	Ø	30.7
2203	RT Alone	~~1.0~~ 1.5x1.5	1.5x1.5x1.5	24.5
~~SAC 2204~~	~~RT Alone~~			
2342	RT Alone	22x19x9	20x15x8	23.5
2343	RT Alone	12x10x4	13x12x6	27.7
2344	RT Alone	Ø	Ø	21.5
2345	RT Alone	Ø	Ø	24.7
2346	RT Alone	Ø	Ø	24.5
did 2347	RT Alone	20x18x9	[illegible]	
2348	RT Alone	15x11x6	15x12x6	20.1
~~SAC 2349~~	~~RT Alone~~			
2350	RT Alone	8x7x3	8x6x4	27.5
2351	RT Alone	10x7x3	9x7x4	26.0
2352	RT Alone	10x9x2	8x8x2	28.7
2353	RT Alone	8x5x2	8x5x3	26.7
2687	RT Alone	11x8x5	9x8x5	25.1
2688	RT Alone	10x9x4	10x9x5	28.3
2689	RT Alone	12x12x9	15x12x12	24.3
2690	RT Alone	9x6x3	7x6x3	28.5
2679	10^7 3636RT	Ø	Ø	24.9
~~SAC 2680~~	~~10^7 3616RT~~			
2681	10^7 3636RT	12x8x5	12x9x5	23.3
~~2682~~	~~10^7 3616RT~~			
2691	10^7 3636RT	7x5x1.5	Ø	23.1
2692	10^7 3616RT	~~[illegible]~~ Ø	6x6x1.5	24.0
2693	10^7 3636RT	7x7x1.5	8x6x1.5	24.6
2694	10^7 3616RT	10x7x4	10x7x3	26.6
2683	10^7 3616RT	Ø	Ø	24.6
2684	10^7 3636RT	Ø	Ø	22.6
2685	10^7 3616RT	Ø	Ø	.4
2686	10^7 3616RT	4x3x1.5	1.0x1.0x1	.2
2675	10^7 8996RT	4x4x1.5 ~~[illegible]~~	4x4x1.5	.3
2676	10^7 8996RT	~~[illegible]~~ 8x8x4	9x7x4	20.3
2677	10^7 8996RT	1.5x1.5x1.5	6x4x1.5	24.7
2678	10^7 8996RT	9x8x3	8x6x3	21.5

HISTO-PATHOLOGICAL

	3d	6d	9d
Control			
RT alone			
RT + 3616			
3616 Alone			

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	2671	10^7 8996RT	Ø	Ø	24.5
	2672	10^7 8996RT	Ø 1x1x1	Ø 1x1x1	28.7
	2673	10^7 8996RT	6x5x1.5	6x3x1.5	22.7
	2674	10^7 8996RT	1.5x1.5x1.5	1.5x1.0x1.0	24.3
	2695	10^7 8996RT	5x5x3	0x4x2	24.9
	2696	10^7 8996RT	11x8x5	12x12x6	21.3
	2697	10^7 8996RT	7x6x3	7x5x2.5	25.3
	2698	10^7 8996RT	14x12x7	10x9x5	15.1
	2663	899-6	17x15x8	20x16x8	22.6
	~~SAC [illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
	~~SAC [illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
	~~SAC [illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
	2703	899-6	21x21x5	21x21x7	25.9
	2704	899-6	20x18x5	20x16x6	26.9
	~~SAC 2706~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
dnd	~~2706~~	~~899-6~~	~~[illegible]~~	~~[illegible]~~	
	2711	899-6	18x15x8	14x17x10	24.2
	2712	899-6	15x12x8	15x13x8	26.7
	2713	899-6	15x12x9	16x16x8	22.9
	2714	899-6	15x15x9	15x15x7.5	24.4
SAC	2707	3616	19x17x11	23x20x14	27.5
	2708	3616	14x10x10	16x14x10	25.2
SAC ★	2709	3616	24x23x11	—	
	2710	3616	14x17x12	19x17x12	25.2
	2719	3616	Ø	Ø	22.9
	2720	3616	Ø 10x10x7	11x9x6	24.6
	2721	3616	16x14x6	17x17x8	28.4
	2722	3616	Ø	Ø	26.0

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2342	RT Alone	20x14x8	20x14x8	26.2
2343	RT Alone	14x10x7	12x11x7	22.1
2344	RT Alone	Ø	Ø	20.30
2345	RT Alone	Ø	Ø	25.2
2346	RT Alone	Ø	Ø	25.7
SAC 2347	RT Alone			
2348	RT Alone	14x14x6	16x14x6	21.6
SAC 2349	RT Alone			
2350	RT Alone	10x8x4	7x6x3	28.1
2351	RT Alone	9x6x2	8x7x3	26.2
2352	RT Alone	11x7x2	9x7x3	28.7
2353	RT Alone	7x6x2	7x5x2	26.1
2687	RT Alone	9x7x4	10x7x4	24.7
2688	RT Alone	10x7x4	10x10x4	29.0
2689	RT Alone	13x11x3	13x12x5	23.6
2690	RT Alone	8x6x3	8x6x3	27.9
2679	10^7 3636RT	Ø	Ø	25.2
SAC 2680	10^7 3616RT			
2681	10^7 3636RT	12x10x6	12x10x9	22.6
SAC 2682	10^7 3616RT			
2691	10^7 3636RT	Ø	Ø	23.3
2692	10^7 3616RT	6x6x1.5	6x5x1.5	30.3
2693	10^7 3636RT	6x5x1.5	5x4x1.5	25.2
2694	10^7 3616RT	8x6x2	8x6x2	26.8
2683	10^7 3616RT	Ø	Ø	24.6
2684	10^7 3636RT	Ø	Ø	22.4
2685	10^7 3616RT	Ø	Ø	26.4
2686	10^7 3616RT	1.0x1.0x1.0	Ø	28.4
2675	10^7 8996RT	4x4x1.5	4x4x1.5	27.8
2676	10^7 8996RT	10x8x4	[illegible]x9x6	21.6
2677	10^7 8996RT	4x3x1.5	6x4x1.5	23
2678	10^7 8996RT	8x6x3	7x6x2	28.6
2671	10^7 8996RT	Ø	Ø	24.4
2672	10^7 8996RT	Ø	Ø	27.8
2673	10^7 8996RT	5x5x1.5	4x4x1.5	26.0
2674	10^7 8996RT	Ø	Ø	24.5
2695	10^7 8996RT	3x3x1.0	2x2x1.5	24.5
2696	10^7 8996RT	11x10x6	11x10x4	23.0
2697	10^7 8996RT	7x5x2	6x4x2	26.0

[redacted]

2698	10^7 8996RT	10 x 7 x 6	10 x 7 x 5	16.5
SAC [redacted] 2683	899-6	21 x 19 x 13		
SAC 2684	899-6			
SAC 2665	899-6			
SAC 2666	899-6			
SAC [redacted] 2703	899-6	24 x 23 x 11		
SAC [redacted] 2704	899-6	20 x 16 x 7	20 x 18 x 11	
SAC 2705	899-6			
SAC 2706	899-6			
SAC [redacted] 2711	899-6	22 x 20 x 7		
Dic [redacted] 2712	899-6	10 x 13 x 10		
SAC [redacted] 2713	899-6	21 x 20 x 13		
2714	899-6	18 x 15 x 8	17 x 14 x 14	25.7
SAC 2707	3616			
Dic [redacted] 2708	3616	12 x 12 x 13		
SAC 2709	3616			
Dic [redacted] 2710	3616	19 x 18 x 14		
2719	3616	0	0	23.8
2720	3616	0	0	25.9
SAC 2721	3616	22 x 20 x 9		
TI 2722	3616	13 x 12 x 6	14 x 12 x 6	24.6

	pre-tx	
2731	9 x 8 x 7	
~~2732~~ 3616 + RTX + GANC	~~[illegible]~~	
2733	13 x 12 x 9	
~~2734~~		

2715	15 x 15 x 12	
2716	20 x 17 x 7	
2717	14 x 10 x 6	
2718	18 x 14 x 10	

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2346	RT Alone	φ	φ 2 25.3	φ
SAC 2347	RT Alone	—	—	
2348	RT Alone	16x14x7	17x14x8 21.7	
SAC 2349	RT Alone		—	
2350	RT Alone	9x7x3	8x6x3 22.3	
2351	RT Alone	8x6x3	8x7x3	25.8
2352	RT Alone	7x7x2	10x8x3	28.1
2353	RT Alone	7x5x3	7x5x2	26.3
2687	RT Alone	9x8x5	10x8x3	25.8
2688	RT Alone	11x10x4	11x9x4	28.9
2689	RT Alone	15x12x4	16x13x5	27.8
2690	RT Alone	8x6x3	7x5x3	28.2
2679	10^7 3636RT	φ	φ	25.3
SAC 2680	10^7 3616RT	—	—	
2681	10^7 3636RT	13x12x9	13x12x8	21.0
SAC 2682	10^7 3616RT	—	—	
2691	10^7 3636RT	6x5x1.5	6x4x1.5	29.2
2692	10^7 3616RT	φ	φ	22.9
2693	10^7 3636RT	4x4x1.5	4x3x1.5	24.7
2694	10^7 3616RT	6x5x2	6x6x2	27.0
2683	10^7 3616RT	φ	φ	24.0
2684	10^7 3636RT	φ	φ	23.3
2685	10^7 3616RT	φ	φ	26.2
2686	10^7 3616RT	1x1x1	φ	22.1
2675	10^7 8996RT	6x6x2	5x5x2	27.5
2676	10^7 8996RT	9x9x4	10x9x5	21.4
2677	10^7 8996RT	5x3x1.5	5x3x1.5	24.2
2678	10^7 8996RT	6x5x2	6x5x1.5	29.1
2671	10^7 8996RT	φ	φ	23.8
2672	10^7 8996RT	φ	φ	27.8
2673	10^7 8996RT	4x2x1.5	4x1.5x1.5	26.2
2674	10^7 8996RT	φ	φ	24.5
2695	10^7 8996RT	1x1x1	1x1x1	28.5
2696	10^7 8996RT	11x10x6	10x9x5	23.0
2697	10^7 8996RT	7x5x2	8x5x3	25.3
2698	10^7 8996RT	11x8x5	12x10x4	17.1
dead 2714	899-6	18x16x13	—	

2719	3616	φ	φ	23.9
2720	3616	9x3x1.5	8x4x2	27.2
SAC 2721	3616	~~15x15x6~~	—	
2722	3616	15x15x6	15x15x6	24.9
2715	HSV/ GAN	18x15x11	~~dead sac~~	dead 8/23
2716	HSV/ GAN	21x18x10	23x18x15	21x17x11 21.8
2717	HSV/ GAN	14x11x8	16x13x7	25.9
2718	HSV/ GAN	22x18x10	23x18x15	25.6
2731	HSV/RT/GAN	6x6x1.5	6x6x3	23.4
2733	HSV/RT/GAN	13x13x4	12x11x3	23.6

Data Sheet

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2346	RT Alone	Ø	
SAC 2347	RT Alone	[illegible]	
2348	RT Alone	14x14x7	
SAC 2349	RT Alone	[illegible]	
2350	RT Alone	7x6x3	
2351	RT Alone	7x7x3	
2352	RT Alone	8x7x5	
2353	RT Alone	7x4x3	
2687	RT Alone	10x8x4	
2688	RT Alone	9x6x4 x	
2689	RT Alone	18x15x8	
2690	RT Alone	6x6x2	
2679	10^7 3636RT	Ø	
SAC 2680	10^7 3616RT	[illegible]	
2681	10^7 3636RT	13x11x7	
SAC 2682	10^7 3616RT	[illegible]	
2691	10^7 3636RT	Ø	
2692	10^7 3616RT	4x3x1.5	
2693	10^7 3636RT	1x1x0.5	
2694	10^7 3616RT	3x3x1.5	
2683	10^7 3616RT	Ø	
2684	10^7 3636RT	Ø	
2685	10^7 3616RT	Ø	
2686	10^7 3616RT	Ø	
2675	10^7 8996RT	4x3x2	/
2676	10^7 8996RT	8x8x3	/
2677	10^7 8996RT	4x3x1.5	
2678	10^7 8996RT	1x1x0.5	
2671	10^7 8996RT	Ø	
2672	10^7 8996RT	Ø	
2673	10^7 8996RT	1x1x0.5	
2674	10^7 8996RT	Ø	
2695	10^7 8996RT	Ø	
2696	10^7 8996RT	9x8x3	
2697	10^7 8996RT	6x6x3	
2698	10^7 8996RT	12x10x5	
SAC 2714	899-6	[illegible]	

2719	3616	0	
2720	3616	6x6x3	
SAC 2721	3616	[illegible]	
2722	3616	16x15x6	
SAC ~~[illegible]~~	~~HSV/ GAN~~		
2716	HSV/ GAN	18x16x8	
2717	HSV/ GAN	18x13x7	
SAC 2718	HSV/ GAN		
2735	injected 8/28/15	14x10x6	
2731	HSV/RT/GAN	5x4x3	
2733	HSV/RT/GAN	10x10x3	

(L) 2732 injected 9x9x6

Greg's Mouse	Data	1 1		
Mouse#	Group	Measurements	Weight	
2687	RT Alone	11x9x3	25.5	9x7x3
2688	RT Alone	8x8x3	29.3	7x6x3
2689	RT Alone	19x17x8	27.8	20x18x6
2690	RT Alone	8x6x3	28.5	9x5x2
2679	10^7 3636RT	0	15.6	ϕ
SAC 2680	10^7 3616RT			—
2681	10^7 3636RT	13x13x7	20.7	13x13x8
SAC 2682	10^7 3616RT			—
2691	10^7 3636RT	0	23.5	ϕ
2692	10^7 3616RT	0	30.2	ϕ
2693	10^7 3636RT	0	25.1	ϕ
2694	10^7 3616RT	9x4x2	25.8	9x7x2
2683	10^7 3616RT	0	25.5	ϕ
2684	10^7 3636RT	0	23.7	ϕ
2685	10^7 3616RT	0	26.7	ϕ
2686	10^7 3616RT	0	21.8	ϕ
2675	10^7 8996RT	5x5x3	29.5	4x4x3
2676	10^7 8996RT	8x6x3	23.7	8x6x4
2677	10^7 8996RT	5x3x1.5	24.4	4x2x1.5
2678	10^7 8996RT	1x1x0.5	30.4	1x1x0.5
2671	10^7 8996RT	0	24.8	ϕ
2672	10^7 8996RT	0	21.2	ϕ
2673	10^7 8996RT	1x1x0.5	22.8	ϕ
2674	10^7 8996RT	0	25.4	ϕ
2695	10^7 8996RT	1x1x1	24.7	4x4x1
2696	10^7 8996RT	8x8x3	23.7	9x6x2
2697	10^7 8996RT	11x9x5	26.2	13x9x6
2698	10^7 8996RT	13x11x6	20.0	13x13x5
2719	3616	0	24.3	ϕ
2720	3616	7x7x3	28.4	10x9x6
SAC 2721	3616			
2722	3616	19x15x6	26.6	20x18x8
SAC 2715	HSV/ GAN			
2716	HSV/ GAN	20x16x10	21.9	20x16x8
2717	HSV/ GAN	24x18x10	26.7	24x20x13
SAC 2718	HSV/ GAN			

1

Data Sheet

injured	2735	HSV/ GAN			18x13x10
	2731	HSV/RT/GAN	5x3x2	23.5	5x3x1.5
	2733	HSV/RT/GAN	9x9x0.5	24.8	6x6x0.5
injured	2732	HSV/RT/GAN			11x10x7

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2687	RT Alone	9x6x3	26.1	7x5x2
2688	RT Alone	7x5x2	28.8	6x6x4
2689	RT Alone	19x16x6	24.5	18x14x6
2690	RT Alone	9x6x4	29.7	6x5x2
2679	10^7 3636RT	0	25.8	0
SAC 2680	10^7 3616RT			
2681	10^7 3636RT	15x13x8	20.7	15x14x8
SAC 2682	10^7 3616RT			
2691	10^7 3636RT	0	24.2	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3636RT	0	26.0	0
2694	10^7 3616RT	9x7x2	25.0	10x8x4
2683	10^7 3616RT	0	25.8	0
2684	10^7 3636RT	0	23.8	0
2685	10^7 3616RT	0	27.2	0
2686	10^7 3616RT	0	22.2	0
2675	10^7 8996RT	5x4x3	29.7	4x3x2
2676	10^7 8996RT	6x6x4	24.8	5x5x1.5
2677	10^7 8996RT	0.5x0.5x0.5	25.7	0.5x0.5x0.5
2678	10^7 8996RT	1x1x0.5	30.5	0.5x0.5x0.5
2671	10^7 8996RT	0	24.8	0
2672	10^7 8996RT	0	28.3	0
2673	10^7 8996RT	0	27.6	0
2674	10^7 8996RT	0	26.5	0
2695	10^7 8996RT	7x5x2	26.2	8x6x3
2696	10^7 8996RT	9x6x3	23.4	5x3x1.5
2697	10^7 8996RT	13x11x6	27.0	15x10x7
2698	10^7 8996RT	15x15x5	20.0	15x15x6
2719	3616	0	25.3	0
2720	3616	10x10x7	27.3	11x10x6
SAC 2721	3616			
SAC 2722	3616	19x21x10	—	
SAC 2715	HSV/ GAN			
2716	HSV/ GAN	21x18x8	22.9	23x18x10
SAC 2717	HSV/ GAN			
~~2718~~	~~HSV/~~ GAN			
2735		18x12x8	29.7	16x11x6

~~273[illegible]~~	~~HSV/GAN~~			
2731	HSV/RT/GAN	3x2 x 1.5	25.6	9x2x1.5
2733	HSV/RT/GAN	5x5 x 1.5	25.8	0
2732	HSV/RT/GAN	13 x 10 x 8	22.0	12x8x6

Data Sheet

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2687	RT Alone	7x5x2	26.4	6x5x1.5
2688	RT Alone	18x17x7	24.6	20x18x8
2689	RT Alone	6x6x4	30.2	5x5x4
2690	RT Alone	6x4x2	28.1	6x4x2
2679	10^7 3636RT	0	26.3	0
SAC 2680	10^7 3616RT	[illegible]	[illegible]	
2681	10^7 3636RT	16x11x6	21.7	14x13x7
SAC 2682	10^7 3616RT	[illegible]	[illegible]	
2691	10^7 3636RT	0	25.1	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3636RT	0	26.8	0
2694	10^7 3616RT	10x9x6	24.7	12x10x6
2683	10^7 3616RT	0	26.1	0
2684	10^7 3636RT	0	24.0	0
2685	10^7 3616RT	0	27.6	0
2686	10^7 3616RT	0	22.4	0
2675	10^7 8996RT	5x4x3	29.3	5x5x1
2676	10^7 8996RT	0	25.4	0
2677	10^7 8996RT	0.5x0.5x0.5	25.3	0.5x0.5x0.5
2678	10^7 8996RT	0.5x0.5x0.5	30.1	0
2671	10^7 8996RT	0	25.2	0
2672	10^7 8996RT	0	28.8	0
2673	10^7 8996RT	0	28.0	0
2674	10^7 8996RT	0	26.0	0
2695	10^7 8996RT	7x6x3	25.2	7x5x2
2696	10^7 8996RT	0	23.0	0
2697	10^7 8996RT	14x10x6	21.3	14x10x6
2698	10^7 8996RT	15x13x7	19	16x14x4
2719	3616	0	25.1	0
2720	3616	13x12x5	28.4	14x11 5
SAC 2721	3616	[illegible]	[illegible]	
SAC 2722	3616	[illegible]	[illegible]	
2715	HSV/ GAN	[illegible]	[illegible]	
2716	HSV/ GAN	12x10x5	26.7	12x6x4
2717	HSV/ GAN	[illegible]	[illegible]	
2718	HSV/ GAN	[illegible]	[illegible]	

SAC 2735	HSV/ GAN	24x21x14	24.3	
2731	HSV/RT/GAN	0.5x0.5x1.5	26	0
2733	HSV/RT/GAN	0	23.7	0
2732	HSV/RT/GAN	11x9x6	23.2	10x9x5

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Cytokine
Mouse log

Mouse #

5001 cytokine study C.G. (B) ; HSV-1 3616 in (L) tumor ; [illegible] virus in (L) tumor ; irradiate (R) limb 20 Gy ; [illegible]
5002 " " ; HSV-1 3616 in (L) [illegible] ; [illegible] irradiation ; sac day [illegible] ; [illegible]
5003 " " ; HSV-1 3616 in R+L ; [illegible] irradiation ; sac day [illegible] ([illegible])
5004 " " ; HSV-1 3616 in R+L ; irradiate (R) 20 Gy ; sac day [illegible]
5005 " D54 ; HSV-1 3616 in (L) tumor ; [illegible] in (L) ; sac day 1
5006 " D54 ; HSV-1 3616 in (R)+(L) ; 20 Gy [illegible] ; sac day 14
5007 " D54 ; " "
5008 " D54 ; HSV-1 3616 in R+L ; sac day 1
5009 " " ; sac day 1
5010 " "

U-87 culture start up

Purpose: Start U-87 culture c̄ frozen cell line [illegible] for injection into mice

Media: prepare culture medium c̄ [illegible] — Cells: U87 [illegible] 8/21/85
500 cc DMEM
50 cc FBS (10%)
5 cc pen/strep
5 cc Na pyruvate
5 cc NEAA

Methods:

Place frozen cell aliquot into warm H_2O bath
Transfer cells to 5 ml of media in 10 ml conical flask drop by drop
Spin in tabletop for 5' at 800-1000 rpm
Aspirate off supernatant c̄ suction
Resuspend cells in 2 ml of media (drop by drop)
Add 2 ml of cells to 3 mls in T-25 (drop by drop)
Place cells in incubator (top slightly loosened)

C-6 (GBM) in nude mice

Received 4 mice from NCI c C6 (R) in hind legs

	ear tag	(L) tumor	(R) tissue	
5001	ф			injected 4/8/21 in
5002	L			(R) leg
5003	R			injected 4/8/21 in
5004	LR			(R)(L) legs

inject mice c 10 ml 3616 per above

Radiation of C-6 nude

only 2 mice could be radiated due to tumor size
radiated for 20 Gy (10'28")
irradiated ф and ~~[illegible]~~ LR

Harvest C-6 nude mice tumors in 2 mice (day 1)

			(L)	(R)	wt	(L)	(R)
	will harvest	5001	11x9x6 mm	12x7x7	Blank	5.11 g	5.16 g
~~301~~		5002	~~12x7x7~~		tumor + blank	5.64	5.33
~~302~~					tumor	0.53 g	0.17 g
~~303~~		5002	19x15x11 mm	19x15x11 m	Blank	5.17 g	5.14 g
					tumor + blank	7.19	7.12 g
						2.02 g	1.98 g

Day 9 (9/28/95)

days due to tumor size	← 5003	21x18x16	23x20x16	5.15	5.21
				6.64	7.6
				1.49	2.39

Day 9 (9/28/95)

50004	22x16x15	17x14x14	~~5.?~~	~~5.12~~
			5.10	5.01
			7.03	6.60

D54 (GBM) in nude mice - cytokines

Received mice from MD with D54 in (R) hind legs

	ear tag	(L) tumor (mm³)	(R) tumor (mm³)
5005	∅	3x6x6 ∅	14x1x9 3616
5006	L	11x7x6 3616	12x10x6 3616
5007	R	14x9x7 3616	14x11x6 3616

inject mice c̄ 10 ul 3616 (2x10⁵ PFU) per above

Irradiation of D-54 mice

irradiate 3 mice c̄ 20 Gy to (R) hind limb

Harvest of D-54 mice tumors

Harvest 5007 on day 1 (10/5/95); 5005 + 5006 on day 2 (10/18/95)

	(L) mm³	(R) mm³		wt (g) (L)	(R)
5007	14x9x8	16x9x9	tube	5.16	5.03
			tube + tumor	5.93	5.51
			tumor	0.77	0.48
5005	13x10x9	11x6x5	tube	5.18	5.22
			tube + tumor	6.30	5.54
			tumor	1.12	0.32
5006	22x15x12	16x12x9	tube	5.18	5.05
			tube + tumor	6.98	6.31
			tumor	1.80	1.26

PrV1110 D.59 Cytotoxic[illegible]

need 0 PFU ⟨ 0 hr; 96 hr ⟨ 0; 24 / 48
1 PFU ⟨ 0 hr; 96 hr ⟨ 0; 24 / 48
10 PFU ⟨ 0 hr; 96 hr ⟨ 0; 24 / 48

1 2 3 4 5 6 7 8 9

need 9 plates plus 1 plate
set up 12 plates

1) Trypsinize Cells
2) Count Cells $127 \times 12 \times 10^4 = 144 \times 10^5 = 14.4 \times 10^6$
resuspend in ~~12 ml~~ 6 ml
3) Add 10 ml media/plate and 1/2 ml cells soln

Infect Plates 4-9 c̄ HSV-1 (1 PFU or 10 PFU)
~90% confluent
i) Count cells in spare plate
$30 \times 10 ml \times 10^4 = 3 \times 10^6$
∴ for 1 PFU want 3×10^6 PFU/plate
for 10 PFU ″ 3×10^7 PFU/plate

$[3616] = 9 \times 10^9$ PFU/ml

3×10^6 PFU/plate $\frac{1 ml}{9 \times 10^9 PFU} = \frac{1 ml}{3 \times 10^3} \left(\frac{1000 \mu l}{1 ml}\right)$
plates 4,5,6
$= \frac{1}{3} \mu l$/plate
3 PFU, not 1 PFU
need 3 plates
set up 9
μl virus
$9 \mu l$ in 29 ml
media

plates 7,8,9 3×10^7 PFU/plate $\frac{1 ml}{9 \times 10^9 PFU} = \frac{1 ml}{3 \times 10^2} \frac{10^3 \mu l}{1 ml}$
$= \frac{1}{3} \times 10 \mu l$/plate
$= 3.33 \mu l$/plate virus
set up 4 $= 13.33 \mu l$ virus in 12 ml media

Add virus 11:30 10/8/95 in 3 ml 29d media

In Vitro 6.27 Cytokines

mock 0 SPFU { 0 hy 1; 24y { 60° 2; 240° 3 }}

0.1 PFU { 0 hy 4; 24y { 60° 5; 240° 6 }}

10 PFU { 0 hy 7; 24y { 60° 8; 240° 9 }}

∴ need 9 plates plus 1 to count cells
∴ 10 plates ⇒ try to set 12 plates

1) Trypsinize cells
2) Count cells 60 X 24 X 10^4 = 14.9×10^6 in ~~[illegible]~~ 6ml
3) Try to plate 2×10^6 cells / plate
4) Add 0.5ml cell soln to 9 ml media / plate

Infect plates 4-9 c̄ HSV-1 ~~(1 PFU or 10 PFU)~~ 0.1 PFU, 1 PFU
~80% confluent
1) Count cells in spare plate ~~47~~ 47~50
$50 \times 10 \times 10^4 = 5 \times 10^6$ cells / plate

infect ~~.1~~ 0.1 PFU (5×10^6 PFU) & ~~10~~ 1 PFU (5×10^7 PFU)

~~2×10^7 PFU/ml~~

7,8,9 tube 9×10^7 PFU/ml 1 PFU 5×10^6 PFU $\left(\frac{1 ml}{2 \times 10^7 PFU}\right) = \frac{2.5}{10}$ ml = .25 ml
= 250 ul / plate
in 3 ml media
2ml in 12ml 750 λ in 9 ml

~~10 PFU 5×10^7 PFU $\left(\frac{1 ml}{9 \times 10^9}\right) = \frac{1}{2} \times \frac{1}{100}$ ml $\times \frac{1000 ul}{1 ml}$~~ = 5 ul in 3 ml

~~make 12 ml~~ add 20 ul virus

4,5,6 0.1 PFU 5×10^5 PFU $\left(\frac{1 ml}{2 \times 10^9 PFU}\right) = \frac{2.5}{100}$ ml $\frac{1000 ul}{1 ml}$ = 25 λ/pl
75 λ in 9 ml

In Vitro U-251

need 0 PFU < 0h / 24h
0.1 PFU < 0h / 24h
1.0 PFU < 0h / 24h

need 9 plates plus 1 plate to count
set up 12 plates

1) Tryp
2) Count cells 148 x 12 x 10^4 = 163 x 10^6 = 16 x 10^6 resuspend in 12 ml
3) Add 10 ml media/plate + 1/2 ml cell soln.

Plates 80-90% confluent
Count cells in 1 plate 28 or 30

$80 \times 12 \times 10^4 = 960 \times 10^4 = 9.6 \times 10^6 = 10 \times 10^6$

will infect c̄ 0.1 PFU and 1 PFU

0.1 PFU $(10 \times 10^5$ PFU$)$ $10 \times 10^5 \text{PFU} \left(\frac{1 \text{ml}}{2 \times 10^7 \text{PFU}} \right) = \frac{5}{100} \text{ml} \; \frac{1000\lambda}{1 \text{ml}} = 50\lambda$

150λ in 9 ml

1 PFU $(10 \times 10^6$ PFU$)$ $10 \times 10^6 \text{PFU} \left(\frac{1 \text{ml}}{9 \times 10^8 \text{PFU}} \right) = \frac{1.1}{10^2} \; \frac{10^3\lambda}{1 \text{ml}} = 11\lambda/\text{plate}$

44λ in 12 ml

Infected at 9^45 am

4) Harvest 4,5 at 9^40 am
5) Harvest plates 1,2 4,5 7,8 at 6^0
6) Plates 3, 6, 9 24^h ? 9^30 Somewhat brown 20-30% ?

In Vitro D54 Cytotoxic Study (cont) from 10/6/95 [redacted]

After 22° (1:00), add 2 ml 7% serum culture media with irradiated 1/30 [redacted]

Cells look round after tx c HSV1 at 1:30 pm [redacted]
RT
9 Gy for plates 2,3, 5,6, 8,9 at 12^{00}
plan to harvest plates in 6° and 24° post tx
using 7'54", D, 250 kV per 6 Gy [redacted]

Harvest

φ rounding — rounding but not confluent — rounding ~40-50% confluent

Harvest plates 1,2 4,5 7,8 at 6° post RT (6^{00} pm)
will harvest remaining 3 plates at 24° (12^{00} 10/10/95)

Harvest plates
* 3, 6, 9 at 24° post RT (12^{00})

φ rounding — 90% confluent rounding — 30% confluent rounding

In Vitro U-87 Cytotoxicity (cont)

Plates infected c 0.01, 1.0 PFU at 11^{30} am
2° post Tx, add 2nd serum⊕ media

RT Tx'd,
Irradiated at 8^{00} am (~20°)
Harvest at 2^{00} pm (6° post RT) and 8^{00} am

12   4,5   2,8
granularity ← similar   cell rounding
balled ⊕

Harvest at 2^{00} 8^{30} am

3   6   9
same   lots of cell rounding + death

D-54 (GBM) in nude mice - cytokine

Receive 3 mice from MIT c̄ D54 in (B) hind legs

	ear tag	(L) mm³		(R) mm³	
5008	∅	14x9x6	(8/16)	[illegible]	(8/16)
5009	L	16x11x8	(8/18)	15x10x12	(8/16)
5010	R	12x7x5	(8/16)	[illegible]	(8/16)

pre-injected mice c̄ 10 uCi 36 K (2x10^6 [illegible]) per [illegible]

Irradiate Mice c̄

Irradiate 3 mice c̄ 20 Gy to (R) hind limbs
* 5009 partially irradiate 2° to tumor size

Sac Mice

		L mm³	R	L (g)	R
Day 1	5008	14x10x9	11x10x9	5.13	5.15
				5.56	5.62
				0.43	.47g
Day 2	5009	17x15x12	18x15x14	5.17	~~5.16~~ 5.1
				6.73	7.06
				1.56	1.98
	5010	12x10x8	11x9x6	5.18	5.03
				5.98	5.39
				0.70	.36

In Vivo 6-77

Repeat of expt. on 10/6/?? (that expt. had ↑ loss of cells even from 0 PFU plate)

also will do protein [illegible] infection

RT 5 hr 6° post

0 PFU ⟨ 0 Gy — 1
9 Gy ⟨ 6° — 2
24° — 3

0.1 PFU ⟨ 0 Gy — 4
9 Gy ⟨ 6° — 5
24° — 6

1.0 PFU ⟨ 0 Gy — 7
9 Gy ⟨ 6° — 8
24° — 9

Plate ~ 2×10^6 cells / 100mm plate
in 10ml media

cells ~ 80% confluent!
$70 \times 10 \times 10^4 = 7 \times 10^6$ cells / plate

<u>1 PFU</u> 7×10^6 PFU $\frac{1 ml}{9 \times 10^9 PFU}$ [illegible]

∴ 4 λ in 12 ml (2%)
3 ml / plate

<u>0.1 PFU</u>
1 ml 1 PFU + 9 ml media (2%)
3 ml / plate

at 10°
infect 2°, then add 7 ml 10% media
RT 6° post infection ~ 4^{15}

Harvest at	10^{15}	1.2	4.5	7.8
		^ 90%	70%	50% 30%
	4^{15}	3	6	9
		^ 90%	50%	30%

w/ Dates

Received from Joany Chou (7/21/94)

- SK-N-SH human neuroblastoma cell cultures
  medium = 10% FCS in DME
- R3616 (HSV 1 (γ ~~1~~34.5)) titer = 6x10^9 PFU/ml
- R899-6 (R3616 + TNF) titer ~ ~~10^9~~ 6x10^9 PFU/ml (titered in Rei's lab 8/5/94)

Gave Joany Chou (7/21/94)

- AT cells & medium
- SCC 61 cells & medium

Vero cells (medium = 5% FCS in DME) received from Elena 7/28/94.

199V media (virus) received 500 cc. Order from Gibco

ATCC HTB 14 [redacted] [redacted]

Current medium for propagation: Eagle's MEM with non-essential amino acids, sodium pyruvate, 1 mM and Earle's BSS, 90%; fetal bovine serum, 10%.

This is one of a number of cell lines derived from malignant gliomas (See also ATCC HTB 16, 17) by J. Ponten and associates from 1966-69 (Acta Pathol. Microbiol. Scand. *74:* 465-486, 1968; Hum. Hered. *21:* 238, 1971). Cultures were established as explants on grid-supported lens paper or gelatin foam with Eagle's minimum essential medium and 10% bovine calf serum as the culture fluid. Trypsinization of the outgrowth or cells attached to the vessel floor with subsequent transfer to standard vessels in growth medium permitted cell line development. A culture at passage 108 was deposited by J. Ponten in July, 1973. Mycoplasma contamination was eliminated in September, 1975.

ATCC HTB 14 (continued)

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-44; Sex-Female; Race-Caucasian; Blood Type-A+.
Grown as: Monolayer; transferred 1:5 weekly.
Morphology: Epithelial-like.
***In Vitro* Cytopathology:** (P120) Consistent with glioblastoma.
Nude mouse: Produces malignant tumor consistent with glioblastoma.

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 122.
Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.
Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	2	3	1	15	21	7	1
Chromosomes:	40	41	42	43	44	45	47

The stemline chromosome number is hypodiploid, the 2S component occurring at 5.4%. Nine markers [t(1q;?3), t(1p22q), t(6p?11q-), t(6q?7p), t(7q;?), del(12q), t(20;1p;9q), t(?8p;?), and M1] were common to most S metaphases. Neither HSR's nor DM's were detected. The line was originated from a female patient. However, all S metaphases were monosomic for the X chromosome.
Viability: 93%.
Culture Medium: Eagle's minimum essential medium with non-essential amino acids, sodium pyruvate and Earle's BSS, 85%; fetal bovine serum, 15%; antibiotic-free.
Isoenzymes: Me-2, 1; PGM_3, 1; PGM_1, 2; ES D, 1; AK1, 1; GLO-1, 1; G6PD, B.
Phenotype Frequency Product: 0.0017.
Sterility: Tests for mycoplasma, bacteria and fungi were negative.
Species: Confirmed as human by isoenzyme analysis.
Note: This material is available under the conditions that you will not use it for commercial purposes or distribute it to third parties. Please see pages xv and xvi for the form required.
Price Code: J

ATCC HTB 15 U-118 MG (Glioblastoma, human)

Current medium for propagation: Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%.

This line is one of a series derived by J. Ponten and associates as discussed under ATCC HTB 14, 16 and 17 (Acta Pathol. Microbiol. Scand. *74:* 465-486, 1968). The source tumor was described as a grade III astrocytoma-glioblastoma with one area resembling an epdendymoblastoma. Cytoplasmic granulation was striking and astroblasts with neurofibrils were observed. Spongioblasts were abundant in culture and were not affected by frequent subcultivation.

A culture at passage 416 was provided originally by J. Ponten. Progeny transferred to the ATCC in 1982 were found to be contaminated with mycoplasma. The infection was cured in 1987 by treatment with BM cycline over a 6-week culture period.

CHARACTERISTICS REPORTED FOR TRANSFERRED STOCK

Patient Data: Age-50; Sex-Male; Race-Caucasian; Blood Type-A+.
Grown As: Monolayer; transferred 1:3.5 weekly.
Morphology: Mixed.
Karyology: Hypopentaploid to hyperpentaploid with abnormalities including breaks (P419).
***In Vitro* Cytopathology:** All spindle giant cells, malignant.
Nude mouse: Yields pleomorphic malignant tumor consistent with glioblastoma multiforme invading muscle.
HLA Cell Line Phenotype: AW 24, 28; B12, W47 (Pollack, *et al.).*

REFERENCE SEED STOCK PREPARED AT ATCC

Number of Serial Subcultures from Tissue of Origin: 443.
Freeze Medium: Culture medium, 95%; DMSO, 5%; antibiotic-free.
Karyology: Chromosome Frequency Distribution 50 Cells: 2n = 46

Cells:	1	1	3	1	2	4	1	4	1	3	4	2	3	3	5	1	2	2	2	1	1	2	1
Chromosomes:	71	99	102	103	104	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	123	125

ATCC CRL 1690 [redacted]

†**Passage Frozen:** 427. **Current medium for propagation:** Eagle's MEM with non-essential amino acids, 1.0 mM sodium pyruvate and Earle's BSS, 90%; fetal bovine serum, 10%. **Additional Information:** This line was derived by L. Hayflick from a glioblastoma multiforma tumor from a 61-year-old male Caucasian. T98G has an indefinite lifespan and is anchorage-independent, but can enter a viable G1-arrested state when crowded or deprived of serum. These cells should be useful for studies on the mechanisms for cessation of proliferation in quiescent cells, and for studies requiring cells synchronized in G1 phase. This is a hyperpentaploid human cell line. The modal chromosome number should be around 128 to 132. The rate of cells with higher ploidies was 1.39%. Fourteen to 16 marker chromosomes were common to most cells. **Reference:** J. Cell. Physiol. *99:* 43-54, 1979. **Submitted by:** G.H. Stein, University of Colorado, Boulder, CO. Price Code: J

ATCC CRL 1691 **C7 (Mouse hybridoma, anti LDL receptors)**

Complete description appears in the Hybridoma Section of the Catalogue (pp. 333-349).

ATCC CRL 1692 **HISM (Smooth muscle, jejunum, human)**

†**Passage Frozen:** 12; PDL 16. **Current medium for propagation:** Dulbecco's modified Eagle's medium, 90%; fetal bovine serum, 10%. **Additional Information:** This cell line was derived from the *muscularis propria* of the jejunum of a normal 35-year-old female patient. It synthesizes collagen and contains actin stress fibers. It contracts in response to the C-terminal octapeptide of cholecystokinin. **Reference:** Proc. Soc. Exp. Biol. Med. *176:* 503-507, 1984. **Submitted by:** M.F. Graham and R.F. Diegelmann, Medical College of Virginia, Richmond, VA. Price Code: J

ATCC CRL 1693 **NFS-5 C-1 (Pre-B lymphoblast, mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 μg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. *129:* 751-758, 1982). **Additional Information:** This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-2SM ecotropic murine leukemia virus. The cells bear a pattern of markers consistent with identification as large pre-B lymphoblasts (*i.e.*, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB–, sIg–, Ia–, and Ly-1+). The cells spontaneously produce both ecotropic and mink cell focus-forming viruses. **Reference:** J. Immunol. *133:* 744-753, 1984. **Submitted by:** W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1694 **NFS-70 C-10 (Pro-B lymphoblast, mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 μg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. *129:* 751-758, 1982). **Additional Information:** This line was derived from a lymphoma arising in an NFS/N mouse inoculated with Cas-NS-7 ecotropic murine leukemia virus. The cells bear a pattern of markers suggestive of pro-B lymphoblasts (*i.e.*, Mac-1+, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB–, sIg–, Ia–, and Ly-1+). The cells appear to be of a very early stage of commitment to B-cell differentiation. **Reference:** J. Immunol. *133:* 744-753, 1984. **Submitted by:** W.J. Davidson and H.C. Morse, III, NIAID, NIH, Bethesda, MD. Price Code: J

ATCC CRL 1695 **NFS-25 C-3 (Pre-B lymphoblast, mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Dulbecco's modified Eagle's medium with HEPES (25 mM), non-essential amino acids at 0.1 mM each, sodium pyruvate (0.5 mM), oxaloacetic acid (1 mM), added glutamine (+2 mM) insulin (0.2 units/ml) and NCTC 109 at 10%. The formulation is similar to Hybri-Care. Transferrin (2 μg/ml), 2 mercaptoethanol (0.05 mM) and 10% fetal bovine serum are also added (J. Immunol. *129:* 751-758, 1982). **Additional Information:** This line was derived from a spontaneously arising lymphoma in an NFS.C58v-1 mouse. The cells bear a pattern of markers consistent with identification as pre-B lymphoblasts (*i.e.*, Ly-17+, Lyb-2+, Ly-5 (B220)+, ThB–, sIg–, Ia–, and Ly-1+). **Reference:** J. Immunol. *133:* 744-753, 1984. **Submitted by:** W.J. Davidson, and H.C. Morse, III, NIAID, NIH, Bethesda, MD.

Price Code: J

ATCC CRL 1696 **McCoy (Mouse)**

†**Passage Frozen:** Unknown. **Current medium for propagation:** Eagle's MEM with non-essential amino acids and Earle's ... 90%; fetal bovine serum, 10%. **Additional Information:** Little descriptive information about the origin of the McCoy ... in the literature. They were first mentioned by Pomerat, *et al.* (Z. Zellforsch. *47:* 158-174, 1957). The cells ... have originated from the synovial fluid in the knee joint of a patient suffering from degenerative arthritis. ... showed that McCoy cells (designated McCoy A) were indeed human cells. However, another subline ... in fact, of mouse origin and possessed marker chromosomes characteristic of strain L mouse ... to be human, but which actually are mouse cells, have been disseminated from laboratory ... Initial interest in McCoy cells followed the demonstration by Gordon and Quan (Proc. ... 1965) and Gordon, *et al.*, (Appl. Microbiol. *23:* 123-129, 1972) that ionizing radiation ... of McCoy cells to infection by chlamydia strains. A culture of the so-called ... for Disease Control, Cell Culture Department, Atlanta, GA in March, 1984. ... not available. The cells have been used to propagate laboratory strains ... The cell line has been satisfactory for chlamydia growth for at

Price Code: J

... receptors)

... (pp. 333-349).

~~sample~~

~~SQ MOI 5 XR 2h extract 24h~~
~~HS " " "~~
~~SQ MOI 0.1 XR 2h "~~
~~HS " " "~~
~~SQ MOI 5 XR 6h "~~
~~HS " " "~~
~~SQ 0.1 "~~
~~HS " "~~
~~5 " "~~
~~" XR 24h~~
~~0.1 "~~
~~"~~
~~" "~~

sample		TNF pg/ml cell	med
SQ20B MOI 5 extract 24h	∅	54	
"	2h	63	255
"	6h	54	240
"	24h	62	194
SQ20B MOI 0.1 "	∅	200	278
	2	134	429
	6	16	312
	24	7	304
HS MOI 5 "	∅	33	1212
"	2	11	1407
"	6	14	1029
"	24	3	1119
HS MOI 0.1	∅	257	1240
"	2	477	1154
"	6	480	1193
"	24	60	1100

median of	
1	240
2	255
3	240
4	194
" 5	278
6	429
7	
" 8	
9	
10	
" 11	
12	
13	
14	
15	
16	

X .2 ml = pg/[redacted] 10^7 cells
2) 2h is best
1) SQ killed
HS not ↑ ?
y — titrate down virus, ↓ time (<24°), ↓ serum
[illegible]

7/27

Decanted CM

confluent x 4 days unfed
1/200 HSV ± XR (3 Gy) 6 hrs later
18 hr decant & feed
80% or 20% of each onto confluent cells

all 1/200 HSV

Ø fed	0			*
Ø fed → subculture	40/100	122/200	200/500	
3Gy fed	0			
3Gy fed → subculture	90/500	157/10	~200/10^4	*
20% Ø CM	0			
20% Ø CM → subculture 24°	79/200	100/100	236/500	cell cycle effect ?
20% 3Gy	0			
20% 3Gy → subculture 24°	200	100/500	250/10^3	>1000/10^4
80% Ø	confluent			
80% Ø → sub 24°	61/200	43/100	162/500	
80% 3Gy	confluent			*
80% 3Gy → sub 24°	253/500	>1000/10^4	~~500~~/10^3	
24h CM Ø → ? sub 24° ? ± XR				
24h CM 3Gy → ? sub 24° ? ± XR				
48h CM Ø	0/200	0/10^3	0/500	*
48h CM 3Gy	0/200	0/10^3	0/500	

no reduce

? toxin vs depletion vs virus vs artifact (over trypsin too confluent)

8/2/93

1) ? does cell density effect PE   2) ? is a new protein made after treatment

	PE		3 Gy
40 λ	300, 500, 10^3, 2×10^3, 5×10^3, 10^4 →		"
4 λ	"		"

on shaker for 1 hr in 1 ml medium

(2) SQ20B 10^5 in T-75 (2)

(3) SQ20B 5×10^5 on p100 – 4 dishes → ^{35}S-methionine

	PE	3 Gy
40	30/300, 30/500, 180/1000, 300/2000, 25/5000, 15/10^4	20/300, 20/500, 30/1000, 100/2000, 10/5000, 25/10^4
4	30/300, 50/500, 100/1000, 120/2000, 780/5000, 20/10^4	10/300, 25/500, 0/2000, 40/5000, 40/10^4

1. What is the relative infectivity of HSV 3616 in SQ-20B compared to Vero cells?

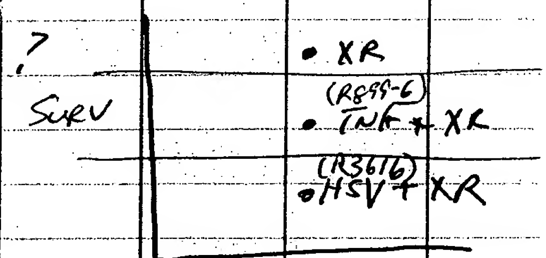
2. What level of ~~tumor~~ viral innocculum can nude mice tolerate?

in vitro

3. At what PFU is TNF production maximized?
At what time point is TNF production maximized?
Compare wt HSV I to HSV 3616 to HSV 899-6
~~Does RT [illegible] TNF production~~

4. How does RT affect cytotoxicity?

where does HSV alone fall?



5. Does supernatant sensitize? How about supernatant to Vero cel
Does supernatant retain infectivity or toxicity?

6. Is TNF produced c̄ viral infection s̄ RT?

7.

AMPAD EFFICIENCY LINE® 22-206

1 Any reason to keep working c̄ R899-6 ?

2

$10\ \mu l + 90\ \mu = 100\ \mu l\ (10^{-1}) + 900\ \mu l\ (10^{-2}) = 1\ ml$ of 10^{-2}

$100\ \mu l\ (10^{-1}) + 900\ \mu l\ (10^{-2}) = 1\ ml$ of 10^{-2}

Need: 199V Glass bottles autoclaved
199Ö " "
Human γ globulin (need to order)

6 T-25's for each cell line
SQ-20B
SK-N-SH
Vero
Need Vero cells from Elena

Reagents
Human γ-glob.
199 V medium
PBS
methanol
Giemsa stain (1X)
Distilled H_2O

Biologicals
6 T-25's of confluent cells p[er] cell type

Equipment
Dilution tubes 1[...]

Need from Elena
- Vero cells
- 199 V media (250[...]

Need to order
- Gamma globuli[n]
ARMOR Pharm

3 Varsene
3 Trypsin
V ½ of V + ½ of T = 6 ml   4 of media
incubate
count c̄ cytometer
? Spin 5/8 for 5 mins
? resuspend c̄ 5% FCS in DME (25 ml)
? 7 #cells per T-25 → incubate (? total vol)
? freeze soln 20% FCS 10% DMSO or Glycerol
? Stock sol'n

13 Gy = 9 ~~36~~ 28
3 ~~52~~
12:28

10^{-5} 10^{-6} 10^{-7} 10^{-8}

~~SQ~~
~~10^{-5}~~
~~10^{-6}~~
~~10^{-7}~~
~~10^{-8}~~

10^{-5} 10^{-6} 10^{-7} 10^{-8}

SQ-20B

SK-N-SH

Vero — —
— —

Doubling times

SQ-20B – 10^5 plated in T-25. On d 4 = 50% confluent

SK-N-SH – 10^5 plated in T-25. On d 4 = 80-90% confluent

Vero cells –

MEDIUM 199/1% calf serum(pH7.6-7.9)

Application: virus propagation and titration.

Preparation: 1X 199v

room T^{o} sterile glass distilled water.............440mls

10X 199 Hank's BSS w/o L-glutamine(Hazleton)....... 50mls

1000X Pn/Strept.....................................0.5mls

heat inactivated bovine calf serum(Colorado Serum).. 5mls

100X L-glutamine(Sigma)............................ 5mls

7.5%(w/v)$NaHCO_3$(Sigma)..............................9.35mls

(1) $NaHCO_3$ must be added last.

(2) $NaHCO_3$ concentration must be 1.4g/l in the 1X formulation. The color of the 1X medium after addition of $NaHCO_3$ should be cherry red. If necessary, add 6N NaOH dropwise to achieve the desired color. DO NOT increase amount of $NaHCO_3$.

(3) Store at 4^{o}C; shelf life 4-6 weeks.

Preparation: 1X 199o

1X 199v...500mls

pooled human gamma globulin.........................0.5mls

To 500mls 1X 199v asceptically add 0.5mls(0.1%) pooled human immunoglobulin.

Shelf life: suggest preparing fresh.

Medium 199 = Gibco Cat No 11181-021
10 X
c̄ Hanks salts
c̄ L-glut
s̄ $NaHCO_3$

GIEMSA STAIN

Application: Solution used to stain viable cells; specifically used in cell culture for virus titration assay.

Source: Sigma

Preparation: 10X GIEMSA

Time Element: 16 day period

giemsa powder..............5 grams
glycerol...................500mls
methanol...................500mls

Day1
(1) Put giemsa and glycerol into a flask which has a layer of glass beads .
(2) Place flask on a shaker at 37°C; shake overnight.

Day2
(3) Remove flask from 37°C and add methanol.
(4) Mix(with stir bar)at room temperature .
(5) Place in dark for 2 weeks(This can be achieved by completely wrapping flask with foil.) at room temperature.

Day16
(6) Filter solution with Whatman #1 paper.
(7) Store at room temperature. No known expiration.

Strengths:

Stock: 10X

Working: 1X (Prepare day of use by diluting 10X stock 1:10 with glass distilled water; eg: 1ml 10X + 9mls water).

use 7.5ml (10 x stock) ~~in 50 ml vol~~ + 42.5 ml dH_2O

SQ20B: 3616 vs 899-6, 10^{-1} vs 10^{-3} MOI

8/3/94-1 SIBLEY

EFFICIENCY LINE® 22-206

Purpose

- Quantify cell killing c̄ R3616 & R899-6 vs. no virus following 5 Gy RT.
- Does the TNF construct in R899-6 lead to additional cell kill over R3616?
- Quantify TNF production in serum and pellet after RT & infection.
- Compare cell killing ~~with~~ and TNF production with MOI of 10^{-1}, 10^{-3}.

Design

- Fixed Variables - Cell type: SQ-20B
  RT dose: 5 Gy single fraction
  Interval infect → RT: 16 hrs
  Interval RT → subculture: 4 hrs

- Study Variables - Virus: None, R3616, R899-6
  MOI: 10^{-1} PFU/cell, 10^{-3} PFU/cell

8/3/94 - 2 SIBLEY

Methods —

8/8/94 ① Plate 10^6 SQ-20B cells / 60 mm dish.
Volume/dish = 3-4 ml 20% FCS in DME + P/S
Incubate to 95% confluency (overnight)
Label Plates: 10^{-1} MOI R3616 10^{-3} MOI R3616
(duplicate) 10^{-1} MOI R3616 10^{-3} MOI R3616
10^{-1} MOI R899-6 10^{-3} MOI R899-6
10^{-1} MOI R899-6 10^{-3} MOI R899-6
Ø virus
Ø virus ∴ need 10 plates + 1 extra to count cells

8/9/94 ② Count a plate using hemocytometer = 1.12×10^6 cell.
③ Make dilution of viruses:
R3616 = 6×10^9 PFU/ml = 6×10^6 PFU/µl

10 µl → 90 → 10 µl → 90 → 10 → 90 → 10 → 90

6×10^5 PFU/µl 6×10^4 6×10^3 6×10^2

R899-6 = 6×10^9 PFU/ml also, ∴ make same dilution.

④ Change media on plates to 2% FCS in DME, 2 ml/pl.
⑤ Add virus (12^{noon})
For 10^{-1} MOI need $\dfrac{1.12 \times 10^5 \text{ PFU}}{6 \times 10^4 \text{ PFU/µl}} = 1.87$ µl of 6×10^4 dilution.

For 10^{-3} MOI need $\dfrac{1.12 \times 10^3 \text{ PFU}}{6 \times 10^2 \text{ PFU/µl}} = 1.87$ µl of 6×10^2 dilution.

(optimally want vol of viral innoculum ~ 2-3 µl)

8/3/94-4

TNF-ELISA RESULTS

1	2	3	4	5	6	7	8	9
R3616		[TNF]	pg/ml				[TNF]	pg/ml
10^{-3}	CM	0			10^{-1}	CM	0	
10^{-3}	CM	0			10^{-1}	CM	0	
10^{-3}	P	0			10^{-1}	P	0	
10^{-3}	P	0			10^{-1}	P	0	
R899-6		[TNF]	pg/ml				[TNF]	pg/ml
10^{-3}	CM	0			10^{-1}	CM	~~25~~	32.7
10^{-3}	CM	0			10^{-1}	CM	~~25~~	32.3
10^{-3}	P	0			10^{-1}	P	~~110~~	51.5
10^{-3}	P	0			10^{-1}	P	~~170~~	56.6
Ø virus		[TNF]	pg/ml					
	CM	0						
	CM	0						
	P	0						
	P	0						

CM = Conditioned media
P = pellet (resuspended @ 10^6 cells/ml)
10^{-3}, 10^{-1} = Viral MOI

ELISA

Standards			
0 med.	1	9	17
15.7	2	10	
33.1	3	11	18
62.5	4	12	
125	5	13	19
250	6	14	
500	7	15	20
1000	8	16	

Put TNF in 1ml distilled H_2O

1. dilute standards in 0.5ml RD5 buffer (1:2)

2. Add standards (+ media only) 200µl to duplicate wells

3. Add 200µl of sample to each well, cover Shake 1 hr.

4. Wash 3X by aspirating liquid, adding ~200µl of wash buffer

5. Add 200µl of conjugate A - shake ~30 min.

6. Wash again (make up A+B)

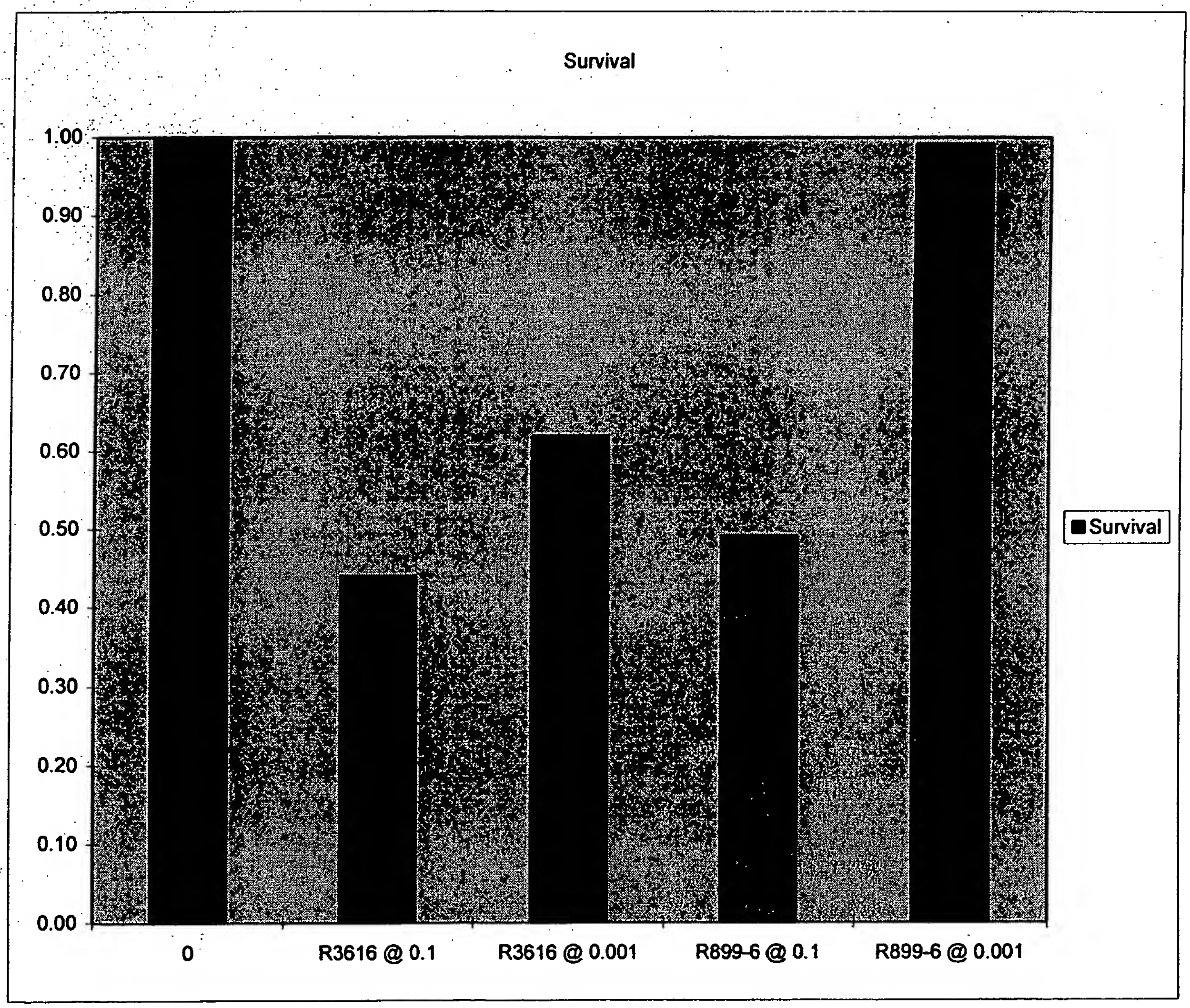
7. A 200µl A+B

1. 3616 10^{-3} CM
2.
3. 3616 10^{-3} P
4.
5. ~~899-6~~ 3616 10^{-1} CM
6.
7. ~~899-6~~ 3616 10^{-1} P
8.
9. 899-6 10^{-3} CM
10.
11. 899-6 10^{-3} P
12.
13. 899-6 10^{-1} CM
14.
15. 899-6 10^{-1} CM

17. Ø v.ns CM
18. ~~[illegible]~~
19. Ø v.ns P
20. ~~[illegible]~~

8/3/94 Results - Counts SQ20B					
RT	Virus	# Plated	Ave. Coun	P.E.	Survival
5	0	1000	176	0.176	1.00
5	R3616 @ 0.1	1000	77.5		0.44
5	R3616 @ 0.001	1000	109		0.62
5	R899-6 @ 0.1	1000	86.5		0.49
5	R899-6 @ 0.001	1000	174.5		0.99
Virus	Survival				
0	1.00				
R3616 @	0.44				
R3616 @	0.62				
R899-6 @	0.49				
R899-6 @	0.99				

SQ



Survival
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
R3616 @ 0.1
R3616 @ 0.001
R899-6 @ 0.1
R899-6 @ 0.001
Survival

8/3/94 - 6

Findings

1.) In SQ-20B:

a. No detectable TNF production @ MOI 10^{-3} $\bar{c}$ 899-6 (+5 Gy)

b. TNF detectable at MOI 10^{-1} (899-6 + 5Gy) with 55 pg/ml in pellet sample & 32 pg/ml in supernatent sample. (pellet sample = 10^6 cells/ml)

c. Despite TNF production, R899-6 did not produce more cell killing than R3616 (@ MOI 10^{-1} + 5 Gy)

d. Both viruses caused increased cell killing at an MOI of 10^{-1}

e. R3616 appeared to give more effective cell killing than R899-6 or Ø virus at MOI of 10^{-3}.

The University of Chicago Departmental Purchase Order

Purchase Order Number
Z 849078
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
American Type Culture Collection
12301 Parklawn Dr.
Rockville (City) | MD (State) | 20852 (Zip Code)
Payment Terms ______ Delivery charge? ☐ Yes ☐ No
Telephone No. (800) 638-6597 FAX No. ______

Ship To
THE UNIVERSITY OF CHICAGO — Dept. Code: 1050
Radiation and Cellular Oncology (Department)
Michael Beckett (Attn:) — G-03 (Room)
5830 S. Ellis Ave.
Chicago (City) | IL (State) | 60637 (Zip Code)

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; second copy should be sent to vendor; third copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with department code number from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ______ Payroll No. ______
Mahmud Ghaemi (Print Name) — 2- (Ext.)
Account Code 5-25754-5400 | 8/8/94 (Date)

Order placed by phone? ☐ No ☒ Yes
Alonzo (Order placed with (name)) | 8/8/94 (Date)
Michael Beckett (Order placed by (name)) | 2-4833 (Phone #)

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	Flask	ATCC HTB 14 U-87 MG Cell Line	75.00	75.00
		Lab Fee	35.00	35.00
		Pkg.-Fee	5.00	5.00
		Fed. Express	9.75	9.75
		Acct. #2168		
			ORDER TOTAL	12[illegible]75

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00

Form ZDPO 100 06/92

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — **PLATE #:** 1

ASSAY :

DATE: 8 / 11 / 94

OPERATOR: SIBLEY — **O.D. LIMIT:** 3.000 — TIME: ____ : ____ AM/PM

WAVELENGTH: 450nm - OPT 2 — **READ MODE:** OPTICAL DENSITY — **AUTO MIX:** OFF

NOTES: — **CAL:** ON

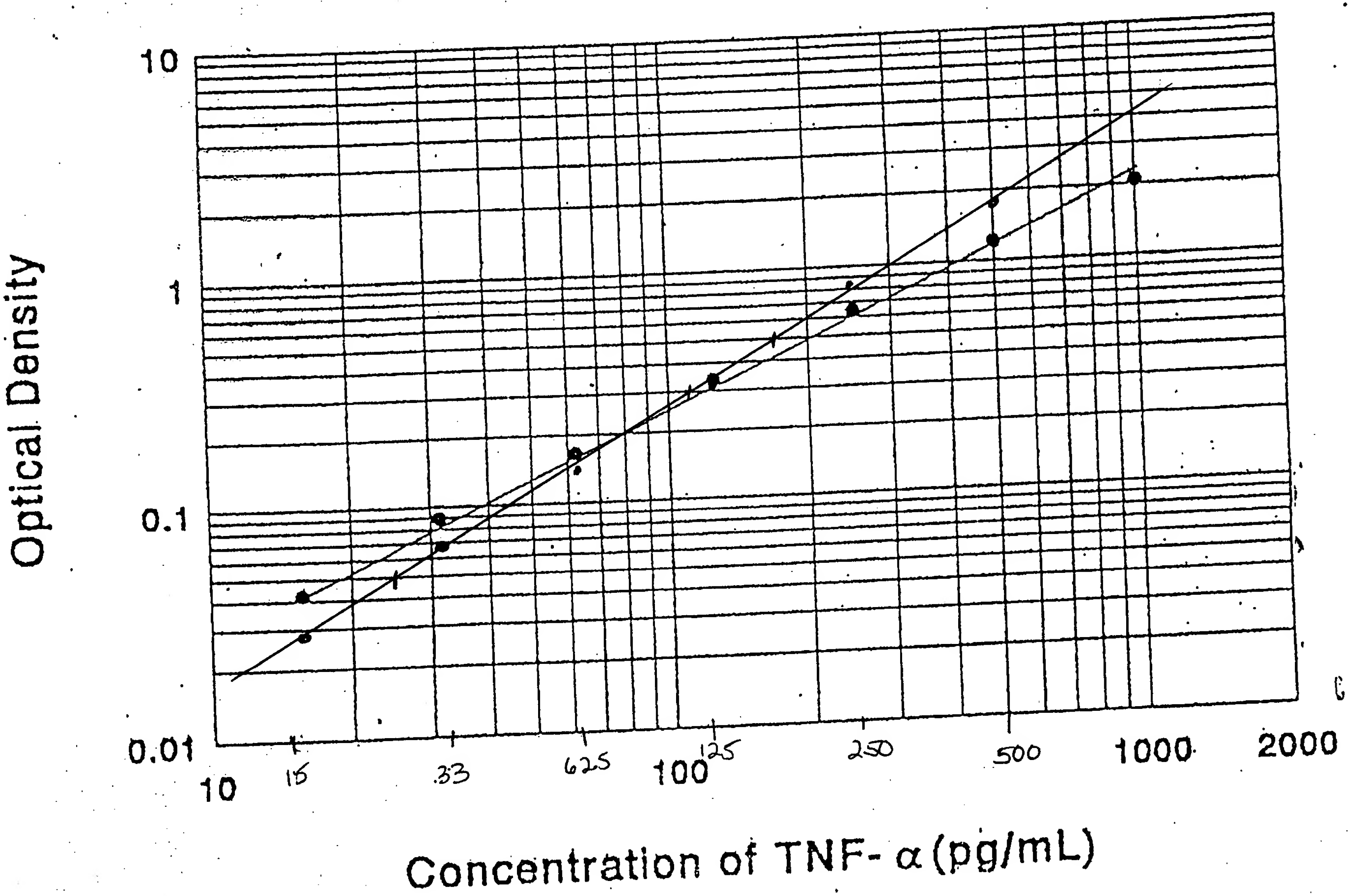
3616 — 899-6 — Ovins

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
				0		0						
A	0.052	0.054	0.049	0.051	0.046	0.047	0.047	0.000	0.000	0.000	0.000	0.000
	.028	.027		~~.050~~ 0		0	0					
B	0.080	0.078	0.045	0.056	0.045	0.048	0.046	0.000	0.000	0.000	0.000	0.000
	.069	[illegible]		0		0						
C	0.121	0.120	0.046	0.044	0.047	0.047	0.051	0.000	0.000	0.000	0.000	0.000
	.161	.158		0	~~.064~~	.009	0					
D	0.213	0.207	0.044	0.051	0.044	0.078	0.039	0.000	0.000	0.000	0.000	0.000
	.311	.326		0		.051						
E	0.363	0.352	0.048	0.048	0.106	0.099	0.061	0.000	0.000	0.000	0.000	0.000
	.879 .860	.844		0		.049	0					
F	0.927	0.896	0.046	0.047	~~0.068~~	0.101	0.047	0.000	0.000	0.000	0.000	0.000
	1.777	1.851		0		.120						
G	1.829	1.803	0.047	0.046	~~0.055~~	0.172	0.050	0.000	0.000	0.000	0.000	0.000
	1.814			0		.139	0					
H	*	*	0.049	0.050	0.185	0.197	0.050	0.000	0.000	0.000	0.000	0.000

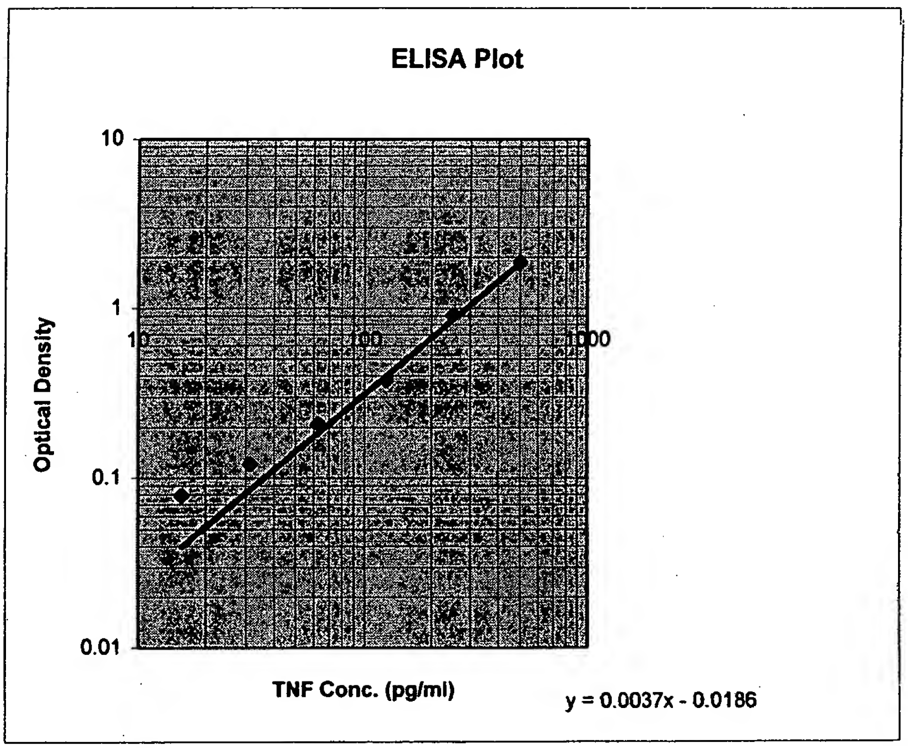
TNF-α Standard Curve

Diluent RD5



ELISA Plotting Worksheet

reading 1	reading 2	concentration	ave. reading
0.08	0.078	15.6	0.079
0.121	0.12	31.3	0.1205
0.213	0.207	62.5	0.21
0.363	0.392	125	0.3775
0.927	0.896	250	0.9115
1.829	1.903	500	1.866
0.106	0.099	32.7	0.1025
0.101	0.101	32.3	0.101
0.172	0.172	51.5	0.172
0.185	0.197	56.6	0.191



T986: 899-6 ~~only~~ @ MOI 10^{-1}
RT 0, 2, 5, 20 [TNF]

8/16/94 -1

SIBLEY

1	2	3	4	5	6	7	8	9

Purpose

- Quantify killing of T986 (glioblastoma cells) with R899-6 virus and 0, 2Gy, 5Gy ~~&~~ 20 Gy RT.
- Determine TNF production (? inducibility) with varying RT doses by ELISA.

Design

- Fixed Variables - Cell type: T986
  ~~RT d~~
  Virus: R899-6 @ MOI of 10^{-1}
  Interval infection → RT: 16 hrs
  Interval RT → subculture: 6 hrs

- Study variables - RT dose: 0, 2Gy, 5Gy, 20 Gy
  Aliquot media for TNF: prior to RT, 30min, 1hr, 2h, 4h,
  Virus: None, R899-6

4 x 2 x 2(dupl) = 16 plates

8/16/94-3

TNF ELISA RESULTS

(Note: error in standards, ∴ old std curve used)

Cell type: T986 (Glioblastoma)
Virus: R899-6 @ MOI of 10^{-1}

RT	Aliquot Time	Virus	[TNF]	
0 Gy	30'	899-6	N/A	
	1°		N/A	
	2°		49	
	4°		64	
	6°		94	
	Pellet (10^5/ml)		23	= 230 @ 10^6/ml
2 Gy	30'	899-6	59	
	1°		67	
	2°		(115)	
	4°		78	
	6°		73	
	Pellet (10^5/ml)		25	= 250 @ 10^6/ml
5 Gy	30'	899-6	46	
	1°		50	
	2°		54	
	4°		70	
	[illegible]		101	
	[illegible]et (10^5/ml)		24	= 240 @ 10^6/ml
20 Gy	30'	899-6	56	
	1°		83	
	2°		66	
	4°		82	
	6°		127	
	Pellet (10^5/ml)		27	= 270 @ 10^6/ml

Controls (No Virus)

$\left.\begin{array}{l} \text{0 Gy} \\ \text{2 Gy} \\ \text{5 Gy} \\ \text{20 Gy} \end{array}\right\}$ No TNF

	reading 1	reading 2	concentration		ave. reading
	0.08	0.078	15.6		0.079
	0.121	0.12	31.3		0.1205
	0.213	0.207	62.5		0.21
	0.363	0.392	125		0.3775
	0.927	0.896	250		0.9115
	1.829	1.903	500		1.866
1°	0.181	0.181	53.9		0.181
	0.143	0.143	43.7		0.143
2°	0.226	0.24	68.0		0.233
	0.2	0.201	59.2		0.2005
	0.291	0.293	83.9		0.292
4°	0.369	0.362	103.8		0.3655
	0.066	0.067	23.0		0.0665
6°	0.069	0.063	22.9		0.066
	0.243	0.253	72.1	30'	0.248
	0.152	0.143	44.9		0.1475
	0.157	0.167	48.8	1°	0.162
	0.303	0.287	84.8		0.295
	0.532	0.517	146.8	2	0.5245
	0.175	0.173	52.1	6	0.174
	0.314	0.336	92.9	6	0.325
	0.203	0.195	58.8	4	0.199
	0.345	0.342	97.9	4	0.3435
	0.293	0.286	83.3	2	0.2895
	0.062	0.067	22.5		0.0645
	0.076	0.086	26.9		0.081

0.157	0.158	47.6	0.1575
0.142	0.146	43.9	0.144
0.148	0.151	45.4	0.1495
0.191	0.179	55.0	0.185
0.183	0.192	55.7	0.1875
0.177	0.17	51.9	0.1735
0.234	0.251	70.6	0.2425
0.231	0.238	68.4	0.2345
0.346	0.337	97.3	0.3415
0.371	0.36	103.8	0.3655
0.063	0.075	23.7	0.069
0.064	0.073	23.5	0.0685
0.182	0.203	57.1	0.1925
0.17	0.193	54.1	0.1815
0.208	0.247	66.5	0.2275
0.348	0.358	100.4	0.353
0.246	0.248	71.8	0.247
0.205	0.209	61.0	0.207
0.261	0.273	77.2	0.267
0.301	0.306	87.1	0.3035
0.457	0.448	127.3	0.4525
0.456	0.437	125.7	0.4465
0.083	0.079	26.9	0.081
0.085	0.079	27.2	0.082

8/16/94 -3

1 STANDARDS		3 0 Gy		5	6	7	8	9
0	med	30'	"	6°	"	2°	"	
15	"	30'	"	6°	"	~~2°~~ 6°	"	
31	"	1°	"	~~30~~ P	"	~~4~~ 6°	"	
62	"	1°	"	P	"	4°	"	
125	"	2°	"	30' $\boxed{2\ Gy}$	"	~~2~~ 4°	"	
250	"	2°	"	30'	"	~~6~~ 2°	"	
500	"	4°	"	~~30~~ 1°	"	P	"	
1000	"	4°	"	1°	"	P	"	
$\boxed{5\ Gy}$								
LANE 9	10	"	12	13	14	15 ØV, NAG		
30'	"	6°	"	2°	"	0Gy-S	"	
30'	"	6°	"	2°	"	0Gy-P	"	
1°		P°	"	4°	"	2Gy-S	"	
1°		P°	"	4°	"	2Gy-P	"	
2°		$\boxed{20\ Gy}$ 30'	"	6°	"	4Gy-S	"	
2°		30'	"	6°	"	4Gy-P	"	
4°		1°	"	P	"	6Gy-S	"	
4°		1°	"	P	"	6Gy-P	"	

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — **PLATE #:** 1

ASSAY : ______________________ DATE: ____/____/____

OPERATOR: ______________________ **O.D. LIMIT:** 3.000 TIME: ____:____AM/PM

WAVELENGTH: 450nm - OPT 2 **READ MODE:** OPTICAL DENSITY **AUTO MIX:** OFF

NOTES: ______________________ **CAL:** ON

OPTICAL DENSITY

	13	14 2	15 3	16 4	5	6	7	8	9	10	11	12
−2° A	0.246	0.248	0.053	0.052	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000
B	0.205	0.209	0.053	0.052	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	0.000
−4° C	0.261	0.273	0.059	0.057	0.000	-0.000	0.000	-0.000	-0.000	0.000	-0.000	0.000
D	0.301	0.306	0.068	0.060	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	0.000
−6° E	0.457	0.448	0.056	0.053	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
F	0.456	0.437	0.055	0.058	-0.001	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
P G	0.083	0.079	0.056	0.058	-0.001	-0.000	-0.000	-0.000	-0.000	-0.001	-0.000	-0.000
H	0.085	0.079	0.059	0.067	-0.001	-0.001	-0.000	-0.000	-0.000	-0.000	-0.001	-0.000

MOLECULAR DEVICES — Vmax KINETICS MICROPLATE READER — **PLATE #:** 2

ASSAY : ______________________ DATE: ____/____/____

OPERATOR: ______________________ **O.D. LIMIT:** 3.000 TIME: ____:____AM/PM

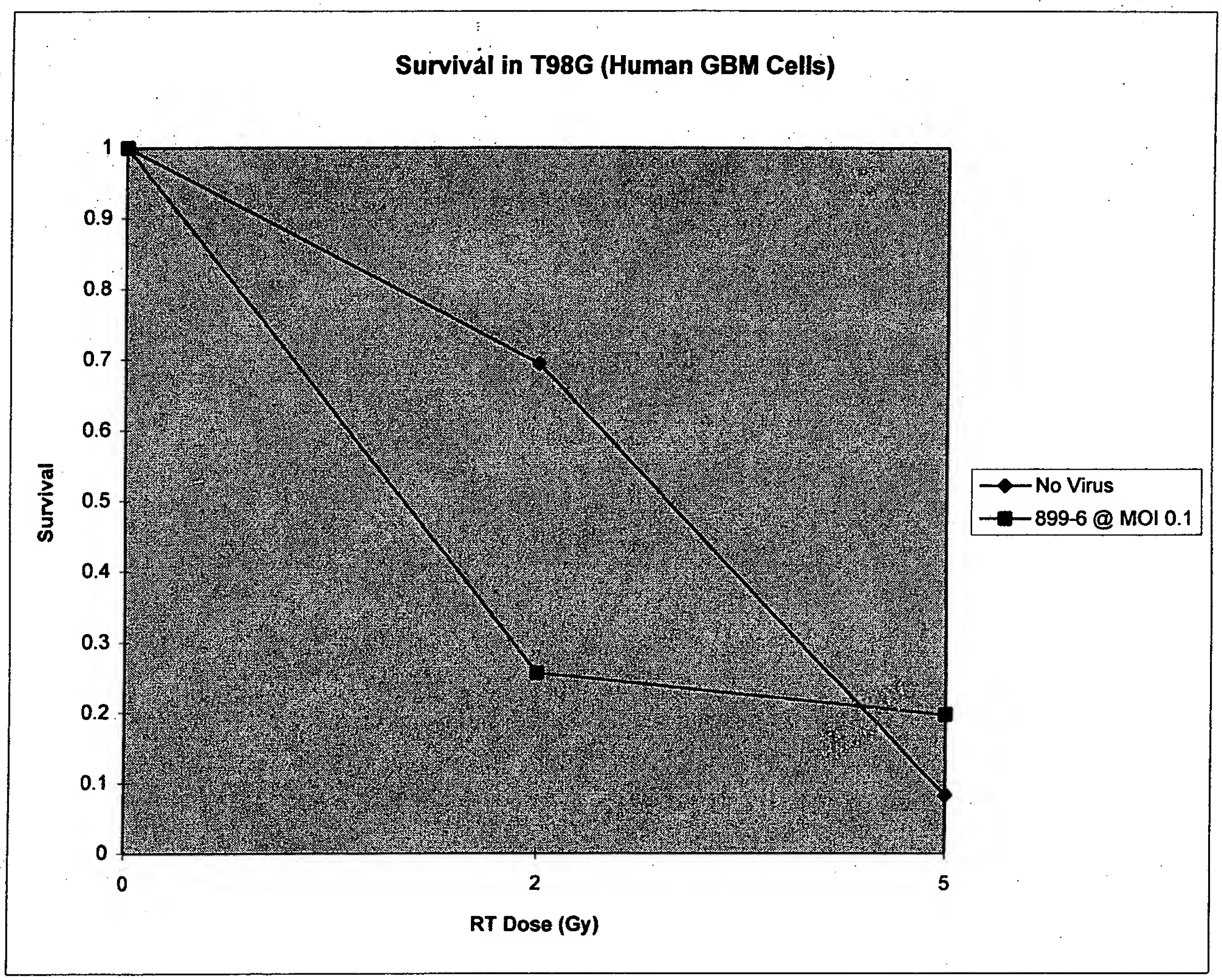
WAVELENGTH: 450nm - OPT 2 **READ MODE:** OPTICAL DENSITY **AUTO MIX:** OFF

NOTES: ______________________ **CAL:** ON

OPTICAL DENSITY

	1	2	3 0Gy	4	5	6	7	8	9 5Gy	10	11	12
A	0.002	0.000	30' -0.000	0.003	6° 0.221	0.253	~~0.522~~	~~0.517~~	30' 0.127	0.158	6° 0.346	0.317
B	0.008	0.006	0.007	0.006	0.369	0.362	0.170	0.173	0.142	0.146	0.37[illegible]	0.350
C	0.004	0.001	1° 0.007	0.004	P 0.066	0.067	6° 0.314	0.336	1° 0.148	0.151	P 0.063	0.075
D	0.000	0.007	0.003	0.007	0.069	0.063	4° 0.203	0.198	0.191	0.179	0.064	0.073
E	0.002	0.006	2° 0.061	0.181	2Gy 30' 2° 0.243	0.253	4° 0.345	0.342	2° 0.183	0.192	30' 20Gy 0.182	0.203
F	0.001	0.004	0.085	0.143	1° 0.152	0.143	2° 0.293	0.288	0.177	0.170	0.170	0.193

5/10



Survival in T98G (Human GBM Cells)
Survival
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
5
RT Dose (Gy)
No Virus
899-6 @ MOI 0.1

8/16/94 Results- Count Plates T98G Cells								
RT	Virus	# Plated	# Counted	#Counted	Average	P.E.	Survival	
0	0	1000	154	104	0.129	0.129	1	
		5000	tm	tm				
		10000	tm	tm				
2	0	1000	75	104	0.0895		0.693798	
		5000	tm	tm				
		10000	tm	tm				
5	0	1000	18	13	0.0155		0.120155	0.081783
		5000	20	44	0.0064		0.049612	
		10000	71	124	0.00975		0.075581	
0	899-6	1000	12	7	0.0095	0.015267	1	
	(MOI=0.1)	5000	68	139	0.0207			
		10000	156	156	0.0156			
2	899-6	1000	3	6	0.0045		0.294118	0.255991
		5000	13	17	0.003		0.196078	
		10000	25	60	0.00425		0.277778	
5	899-6	1000	4	2	0.003		0.196078	0.196078
		5000	18	11	0.0029		0.189542	
		10000	38	24	0.0031		0.202614	
RT Dose	No Virus	899-6 @ MOI 0.1						
0	1	1						
2	0.694	0.256						
5	0.082	0.196						

8/16/94-4

RESULTS - Count plates (9/1/94)

RT	V.rus	# plated	# counted 1	# counted 2	Counted ave.	P.E.	SURVIVAL	AVE SURVIVAL	
Ø	Ø	10^3	154	104	129	.13	100	100	
		5×10^3	Tm	Tm	—	—	—		
		10^4	Tm	Tm	—	—	—		
2 Gy	Ø	10^3	75	104	90	"	69	69	
		5×10^3	Tm	Tm	—	—	—		
		10^4	Tm	Tm	—	—	—		
5 Gy	Ø	10^3	18	13	16	"	12	8.1	
		5×10^3	20	44	32	"	4.9		
		10^4	71	124	98	"	7.5		
					1.7	.017			
Ø	899-6	10^3	12	7	9.5	"	7.3	11.7	
		5×10^3	68	139	103	"	15.9		
		10^4	Tm	156	156	"	12		
2Gy	899-6	10^3	3	6	4.5		3.5	4.3	.17
		5×10^3	23	17	15	"	2.3		
		10^4	25	(160)	25 (92.5)	"	1.9 (7.1)		
5Gy	899-6	10^3	2	4	3	"	2.3	2.3	
		5×10^3	18	11	14.5	"	2.2		
		10^4	38	24	31	"	2.4		

8/16/94 - 5

FINDINGS -

(1) R899-6 virus is highly cytotoxic at an MOI of 10^{-1} to T98G cells with a plating efficiency of only 1.5%.

(2) Higher levels of TNF are produced in T98G cells than in SQ20B cells = 5 fold more at an MOI of 10^{-1} (250 vs 50).

(3) No radiation induction of TNF, i.e. it is produced constitutively.

(4) There is no clear synergistic effect between 5 Gy & 899-6 @ 10^{-1}, although there is possible synergy at 2 Gy.

(5) T98G cells are more radiosensitive than SQ20B cells.

SQ 20B

8/26/94 - 1

Purpose -

- Quantify cell killing of R3616 at MOI of 10^{-2}, 10^{-1}, 5.
- Is killing additive or synergistic $\bar{c}$ 5 Gy?
- Quantify the effect of IgG (human) in the incubation media
- ~~Does the IgG~~ Is there a cell density dependent killing, and does IgG eliminate this phenomenon

Design

- Fixed Variables - Cell type: ~~20~~ SQ 20B
  ~~Virus: R3616~~
  Interval ~~from~~ of infection: 2° ~~then~~
  Interval from infection to RT: 16°
  Interval from RT to subcult: 4-6°

- Study Variables - Virus: None, R3616 @ MOI 10^{-2}, 10^{-1}, 5
  Human IgG: +, -
  RT: 0 Gy, 5 Gy

8/26/94-2
SQ-20B

Virus	moi	IgG	RT	
Ø	—	0	0	5.6
"	"	"	"	4.8
Ø	—	+	0	6
Ø	—	0	5	3.4
"	"	"	"	3.7
Ø	—	+	5	4.2
3616	10^{-2}	0	0	4.2
"	"	"	"	inf.
3616	10^{-2}	+	0	5.9
"	"	"	"	4.2
3616	10^{-2}	0	5	2.7
"	"	"	"	2.4
3616	10^{-2}	+	5	2.8
"	"	"	"	4.2
3616	10^{-1}	0	0	5.9
"	"	"	"	5.8
3616	10^{-1}	+	0	3.3
"	"	"	"	3.35
3616	10^{-1}	0	5	2.2
"	"	"	"	1.9
3616	10^{-1}	+	5	1.8
"	"	"	"	1.8
3616	5	0	0	1.8
"	"	"	"	2.5
3616	5	+	0	
"	"	"	"	
3616	5	0	5	
"	"	"	"	
3616	5	+	5	
"	"	"	"	

switch

need 31 plates × 5 ml = 155 ml
∴ resuspend in 165 ml
10^7 cells in 165 ml = 3×10^5/5 ml

8/26/94 -3

Methods –

8/26/94 (1) Plate 5×10^5 cells per 60 mm dish

8/29/94 (2) Infect $\bar{c}$ virus in 2 ml 2% FCS

Dilutions: Stock $\xrightarrow{10\,\mu l}$ (90) $\xrightarrow{10}$ (90) $\rightarrow$ (90)

6×10^6 PFU/ml 6×10^5 6×10^4 6×10^3

Count dish $= 2 \times 10^6$ cells

– MOI 10^{-2} = $\frac{2 \times 10^4 \text{ PFU}}{6 \times 10^3 \text{ PFU}/\mu l} = 3.3\ \mu l$ of 6×10^3 dilution

– MOI 10^{-1} = $\frac{2 \times 10^5 \text{ cells}}{6 \times 10^4 \text{ PFU}/\mu l} = 3.3\ \mu l$ of 6×10^4 dilution

– MOI 5 = $\frac{1 \times 10^7 \text{ PFU}}{6 \times 10^5 \text{ PFU}/\mu l} = 16.7\ \mu l$ of 6×10^5 dilution

(3) Incubate 2° then ~~add~~ aspirate media &

add: 4 ml of 2% FCS (⊖ IgG) or

4 ml of 2% FCS + human γglob (1 µl/ml) (stock = 165 mg/10...

8/30/94 (4) Irradiate $\bar{c}$ 0 or 5 Gy

(5) Subculture & count each dish $\bar{c}$ hemacytometer

(6) Make dilutions 10^6, 10^5, 10^4, 10^3 for each dish

(7) Plate 3 dilutions for each original dish = 5×10^2, 10^3, 5×10^3

(8) Incubate for 10 d – 14 d

VIRUS	MOI	IgG	RT	# plated	# counted
3616	10^{-1}	–	0	500	96
''	''	+	0	500	101
''	''	+	0	500	251
''	''	–	0	10^3	TM
''	''	+	0	10^3	197
''	''	+	0	10^3	TM
~~''~~	~~''~~	~~–~~	~~0~~	~~5×10^3~~	
~~''~~	~~''~~	~~+~~	~~0~~	~~5×10^3~~	
~~''~~	~~''~~	~~+~~	~~0~~	~~5×10^3~~	
3616	10^{-2}	–	0	500	214
''	''	''	0	500	130
''	''	+	0	500	177
''	''	''	0	500	205
''	''	–	0	10^3	231
''	''	''	0	5×10^3	TM
3616	10^{-2}	–	5	500	49
''	''	''	5	500	43
''	''	+	5	500	25
''	''	''	5	500	48
''	''	–	5	10^3	90
''	''	''	5	10^3	68
''	''	+	5	10^3	47
''	''	''	5	10^3	49
''	''	–	5	5×10^3	Tm
''	''	''	5	5×10^3	Tm
''	''	+	5	5×10^3	205
''	''	''	5	5×10^3	287

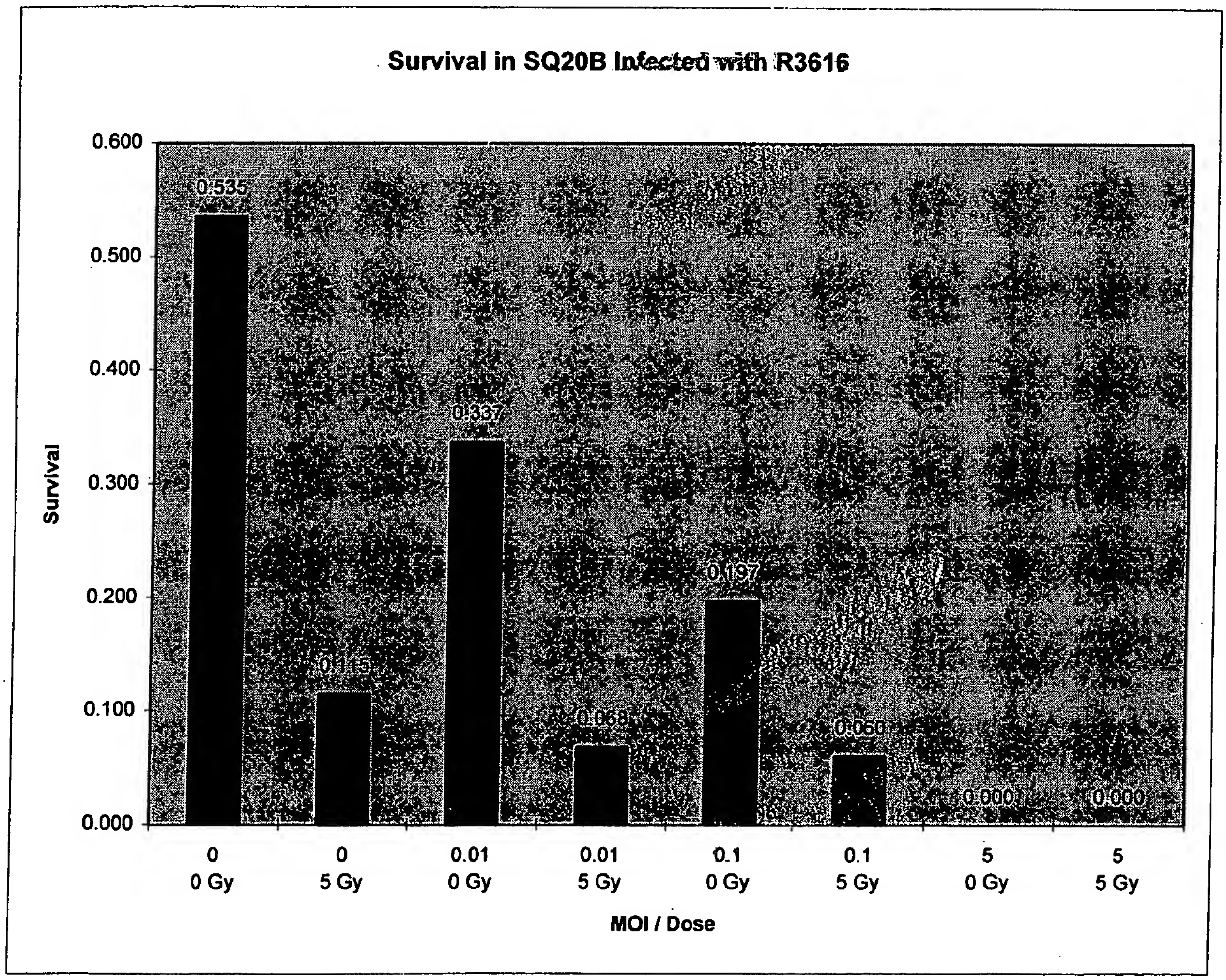
SQ-2013

Virus	moi	IgG	RT	# plated	# counted
0	—	—	0	500	323
		—	0	500	203
		+	0	500	277
				$10^3/5\times10^3$	TM
0	—	—	5	500	36
"	"	"	"	"	72
0	—	—	5	10^3	89
"	"	"	"	"	130
0	"	+	5	5×10^3	TM
0	—	+	5	500	56
"	—	+	5	10^3	140
"	"	"	"	5×10^3	TM
3616	10^{-1}	—	5	500	51
"	"	"	"	500	25
"	"	+	"	500	31
"	"	"	"	500	38
"	"	—	"	10^3	68
"	"	"	"	10^3	55
"	"	+	"	10^3	59
"	"	"	"	10^3	77
"	"	—	"	5×10^3	215
"	"	"	"	5×10^3	185
"	"	+	"	5×10^3	246
"	"	"	"	5×10^3	225

SQ 20B: R3616 BY MOI AND+/- IgG								
Virus	MOI	IgG	RT	# plated	# counte	Crude SV	PE	Rel. SV
0	-	-	0	500	323	0.646	0.535333	100
0	-	-	0	500	203	0.406		
0	-	+	0	500	277	0.554		
0	-	-	5	500	36	0.072	0.533	0.214822
0	-	-	5	500	72	0.144		
0	-	+	5	500	56	0.112		
0	-	+	5	1000	140	0.14		
0	-	-	5	1000	89	0.089		
0	-	-	5	1000	130	0.13		
R3616	0.01	-	0	500	214	0.428	0.3366	100
R3616	0.01	-	0	500	130	0.26		
R3616	0.01	+	0	500	177	0.354		
R3616	0.01	+	0	500	205	0.41		
R3616	0.01	-	0	1000	231	0.231		
R3616	0.01	-	5	500	49	0.098	0.337	0.202733
R3616	0.01	-	5	500	43	0.086		
R3616	0.01	+	5	500	25	0.05		
R3616	0.01	+	5	500	48	0.096		
R3616	0.01	-	5	1000	90	0.09		
R3616	0.01	-	5	1000	68	0.068		
R3616	0.01	+	5	1000	47	0.047		
R3616	0.01	+	5	1000	49	0.049		
R3616	0.01	+	5	5000	205	0.041		
R3616	0.01	+	5	5000	287	0.0574		
R3616	0.1	-	0	500	96	0.192	0.197	
R3616	0.1	+	0	500	101	0.202		
R3616	0.1	+	0	500	[251]	-		
R3616	0.1	-	0	1000	TM	-		
R3616	0.1	+	0	1000	197	0.197		
R3616	0.1	+	0	1000	TM	-		
R3616	0.1	-	5	500	51	0.102	0.197	0.305922
R3616	0.1	-	5	500	25	0.05		
R3616	0.1	+	5	500	31	0.062		
R3616	0.1	+	5	500	38	0.076		
R3616	0.1	-	5	1000	68	0.068		
R3616	0.1	-	5	1000	55	0.055		
R3616	0.1	+	5	1000	59	0.059		
R3616	0.1	+	5	1000	77	0.077		
R3616	0.1	-	5	5000	215	0.043		
R3616	0.1	-	5	5000	185	0.037		
R3616	0.1	+	5	5000	246	0.0492		
R3616	0.1	+	5	5000	225	0.045		
R3616	5	+	0	10000	0	0	0	0
R3616	5	+	5	10000	0	0		

SQ 20B: R3616 BY MOI AND+/- IgG

Dose	MOI	Survival
0 Gy	0	0.535
5 Gy	0	0.115
0 Gy	0.01	0.337
5 Gy	0.01	0.068
0 Gy	0.1	0.197
5 Gy	0.1	0.060
0 Gy	5	0.000
5 Gy	5	0.000



8/26/94

FINDINGS

In SQ-20B:

- There is 63% & 37% survival @ MOI of 10^{-2} & 10^{-1} (corrected for plating efficiency)
- 5 Gy radiation results in 21% survival $\bar{s}$ virus (corrected for plating efficiency)
- Cell killing is <u>additive</u> between virus and radiation at the MOI & dose studied.
- An MOI of 5 results in complete cell killing
- Human γ-globulin in the ~~serum~~ media did not have an effect on survival
- No cell density dependence was observed

V 87 cells

8/29/94-1

EFFICIENCY LINE® 22-206

PURPOSE -

- Compare cytotoxicity of R3616 & R899-6 in V-87 cells (human glioblastoma).
- Determine radiosensitivity of V-87 cells to 5 Gy
- ~~Are~~ Is viral cytotoxicity additive or synergistic with radiation toxicity?
- Quantitate TNF production in R899-6 cells. Does RT (5 Gy) increase TNF production?

DESIGN -

- Fixed variables - cell type: V-87 (human glioblastoma)

Study variables - virus: None, R3616, R899-6

MOI: 10^{-3}, 10^{-1}

RT: 0, 5 Gy

U87 cells

8/29/94-3

1 2 3 4 5 6 7 8 9

Methods

(1) Plate 10^5 cells per 60mm plate, Need 6 plate

9/3/94 (2) When >90% confluent, infect $\bar{c}$ virus. Count cells in extra plate to determine PFU's.

Make dilutions of virus = 6×10^6 PFU/µl (stock) → 6×10^5 → 6×10^4 → 6k

Count dish = 2.65×10^6

$-moi\ 10^{-1} = \frac{2.65 \times 10^5 \text{ PFU}}{6 \times 10^4 \text{ PFU/µl}} = 4.4$ µl

$-moi\ 10^{-3} = \frac{2.65 \times 10^3 \text{ PFU}}{6 \times 10^2 \text{ PFU/µl}} = 4.4$ µl

(3) Incubate 2° in 2% FCS, then subculture dish & make dilutions = 10^6, 10^5, 10^4, 10^3 cells/ml, 10^2

(4) Plate dilutions:

Ø virus, ØRT = 200, 500, 1000

Ø virus, 5 Gy = 5×10^3, 10^4, ~~2~~ $\times 10^4$

virus, ØRT = ~~5×10^2~~, 10^3, 5×10^3, 10^4

virus, 5 Gy = 5×10^3, 10^4, 5×10^4, ~~10^5~~

Media = 10% FCS + P/S + 1% NaPyr + 1% Ess a.a. in MEM $\pm$ γ-96b 1µl/ml

(5) Wait 4°, then irradiate

(6) Incubate

(7) Stain & Count

U-87

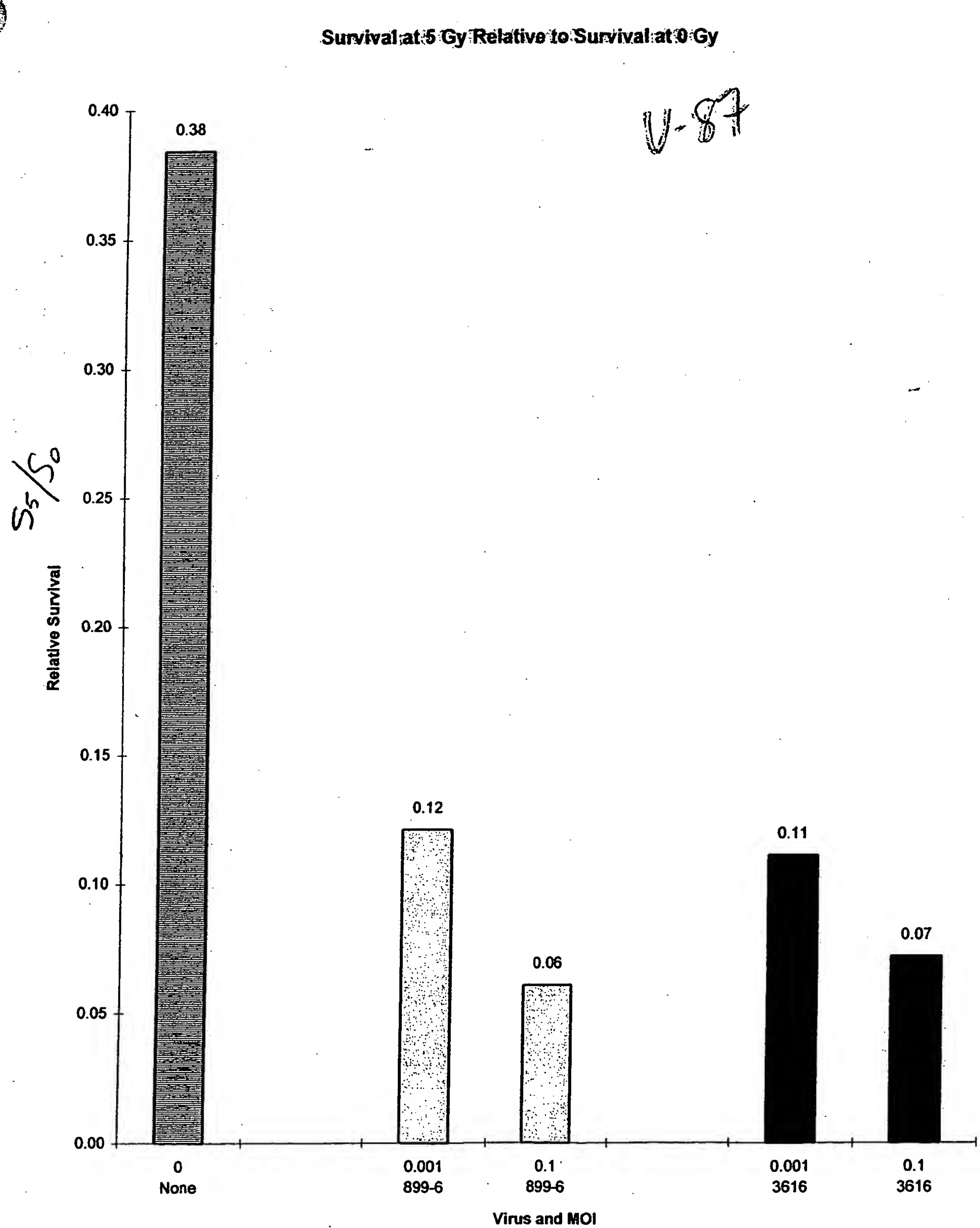
Note: poorly defined plaques, slow growing

8/29/64 - 4

Results -

Virus	MOI	RT	# plated	# counted	Crude SV	PE	Rel SV
∅	–	0	200	0/0			
			500	~~0~~1			
			1000	6			
∅	–	5	5000	1/2			
			10^4	0/12			
			2×10^4	42/45			
899-6	10^{-3}	0	1000	21/6			
			5000	61			
			10^4	?/?			
899-6	10^{-3}	5	5000	1/1			
			10^4	0/7			
			5×10^4	?/106			
899-6	10^{-1}	0	1000	35/6			
			5000	125/69			
			10^4	?/?			
899-6	10^{-1}	5	5000	1/0			
			10^4	6/8			
			5×10^4	138/148			
3616	10^{-3}	0	1000	3/0			
			5000	68/43			
			10^4	?/?			
3616	10^{-3}	5	5000	0/1			
			10^4	12/14			
			5×10^4	?/?			
3616	10^{-1}	0	1000	14/8			
			5000	106/?			
			10^4	?/?			
3616	10^{-1}	5	5000	1/0			
			10^4	4/22			
			5×10^4	?/104(!)			

EFFICIENCY LINE® 22-206 AMPAD


Survival at 5 Gy Relative to Survival at 0 Gy
V-87
S_5/S_0
Relative Survival
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.38
0.12
0.06
0.11
0.07
0
None
0.001
899-6
0.1
899-6
0.001
3616
0.1
3616
Virus and MOI

U-87 8/29/94									
RESULTS:									
Virus	MOI	RT	# plated	# counted		Crude SV	Ave SV	PE	Rel SV
None	-	0	200	0	0	0	0.002667	0.002667	1
None	-	0	500	1		0.002			
None	-	0	1000	6		0.006			
None	-	5	5000	1	2	0.0003	0.001025	0.002667	0.384375
None	-	5	10,000	0	12	0.0006			
None	-	5	20,000	42	45	0.002175			
899-6	0.001	0	1000	21	6	0.0135	0.00735	0.00735	1
899-6	0.001	0	5000	6		0.0012			
899-6	0.001	0	10,000	tm	tm				
899-6	0.001	5	5000	1	1	0.0002	0.00089	0.00735	0.121088
899-6	0.001	5	10,000	0	7	0.00035			
899-6	0.001	5	50,000	106	tm	0.00212			
899-6	0.1	0	1000	35	6	0.0205	0.01995	0.01995	1
899-6	0.1	0	5000	125	69	0.0194			
899-6	0.1	0	10,000	tm	tm				
899-6	0.1	5	5000	0	1	0.0001	0.00122	0.01995	0.061153
899-6	0.1	5	10,000	6	8	0.0007			
899-6	0.1	5	50,000	138	148	0.00286			
3616	0.001	0	1000	3	0	0.0015	0.0063	0.0063	1
3616	0.001	0	5000	68	43	0.0111			
3616	0.001	0	10,000	tm	tm				
3616	0.001	5	5000	0	1	0.0001	0.0007	0.0063	0.111111
3616	0.001	5	10,000	12	14	0.0013			
3616	0.001	5	50,000	tm	tm				
3616	0.1	0	1000	14	8	0.011	0.0161	0.0161	1
3616	0.1	0	5000	106	tm	0.0212			
3616	0.1	0	10,000	tm	tm				
3616	0.1	5	5000	1	0	0.0001	0.00116	0.0161	0.07205
3616	0.1	5	10,000	4	22	0.0013			
3616	0.1	5	50,000	104	tm	0.00208			

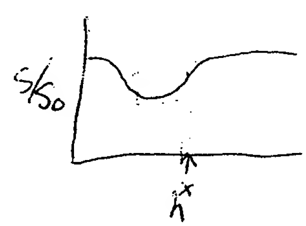
1/2

?

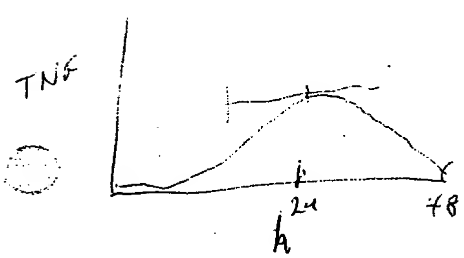
?

variable

time I→S
I→X
S→X



schedule
I→X→S
PLDR
S→I→X



I→ELISA

SQ20B

9/2/94-1

Purpose

- Determine viral cytotoxicity of R3616 @ MOI of 10^{-} & 1 in SQ20B cells
- Is viral cell killing additive or synergistic with 9 Gy RT?

Design

- Fixed variables - Cell type: SQ20B
  ~~Virus~~
  Interval infection → subculture: 2 hours
  Interval subculture → RT: 4 hours
  Human γ globulin added to plates p̄ subculture
- Study variables - Virus: R3616 @ MOI 0, 10^{-1}, 1
  RT: 0, 9 Gy

9/12/94

TITERS

Cell	Virus	Dilution	Plaque #	Titer	Notes
SQ 20B	3616	10^7	0	?	none seen, but cells overgrown. Try titering @ 80% confluence
		"	0		
		10^8	0		
		"	0		
SQ 20B	899-6	10^7	0	?	
		"	0		
		10^8	0		
		"	0		
V-87	3616	10^7	~~39~~ ~~48~~ 8	6×10^7	small plaques
		"	~~42~~ 3		
		10^8	~~8~~ 0		
		"	~~7~~ 0		
V-87	899-6	10^7	~~33~~ 13	~~1.2~~ $\times 10^{8}$	
		"	~~42~~ 8		
		10^8	~~8~~ 0		
		"	~~7~~ 0		
T-986	3616	10^7	14	1.2×10^8	nice plaques
		"	9		
		10^8	0		
		"	0		
T986	899-6	10^7	12	4.1×10^8	
		"	39		
		10^8	3	8×10^8	
		"	7		

THE UNIVERSITY OF CHICAGO ANIMAL RESOURCES CENTER ANIMAL PROCUREMENT REQUEST (#315B)

REQ NO 18773

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Greg Sibley, MD DATE: 10/26/94

REQUESTORS PHONE NUMBER: 312-702-0294

AUTHORIZED SIGNATURE: [illegible]

FAS ACCOUNT: 6-95150-5100

VENDOR: Jackson Labs

REQUESTED DELIVERY DATE: ASAP

P.I.: Hallahan

PROTOCOL: 58671

PHONE: 2-6829

SPECIES: mouse

STRAIN: nu/J

WEIGHT/AGE: 5-7 wks

QUANTITY: 24

SEX: M F EITHER

ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☑ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

HOUSE AT: ___ CARLSON ___ WYLER X CLSC ___ FMI ___ OTHER

T986

10/28/94-1

PURPOSE -

- Establish dose response survival curves with T986 cell line. ~~Dosen~~
- ~~Establish dose response survival curve with T986 cell line + R3616 @ MOI = 10^-1.~~

DESIGN -

- Fixed variables - cell type T986 (human glioblastoma)
  - ~~intervals = infect x 2° → subculture → wait 4° → irradiate~~
- Study variables - virus: None, ~~R3616 @ MOI = 10^-1~~
  RT dose: 0, 2, 3, 5, 7, 9, 11 Gy

TITER ~~CELLS~~ VIRUSES IN 4 cell lines

10/28/94

METHODS -

① Plate cells in T-25's on Friday for use Mon.

- Vero cells plate 10^5/flask
- T986 cells plate 2×10^5/flask
- SQ 20B cells plate ~~3~~ 2×10^5/flask
- U87 cells plated earlier this week

1	2	3	4	5	6	7	8	9

VERO CELLS

		D_1	D_2	D_3
T-25	10^5	30%	40%	sub conf 85-90 -100% ~~conf~~
T-25	2×10^5	50%	80%	
T-25	5×10^5	80-90%	-100%	confl +
T-150	10^5	—	—	

	T986	D_1	D_2	D_3
T-25	5×10^4	-	30%	
T-25	10^5	-	50%	
T-25	2×10^5	30%	70%	

U-87

D_1

10^5

In-Vitro – SQ-20B

1.) Titer virus in SQ-20B
2.) Generate Survival curve for R3616 @ 0, 3, 5, 7, 9, 11 Gy – MOI=0

– U87

1.) Titer virus in U-87
2.) Generate survival curve for R3616 (MOI = 10^{-3}) @ 0, 2, 3, 5, 7 Gy
R3616 (MOI = 10^{-1})
R899-6 (MOI = 10^{-3})
R899-6 (MOI = 10^{-1})

– T98G

1.) Titer virus in U-87
2.) Generate survival curves as above for U87

TNF

1.) Measure in U-87 & T98G infected c̄ R3616 to R/O cellular ~~viral~~ TNF production in response to virus
2.) Quantify

10/31/94

Cell	Virus	Dilution	Plaque	ct	Titer	
Titers						
Vero	3616	10^6	TM	TM		
		10^7	230	bad flask		
		10^8	23	24	2.3 x 10^9	
Vero	899-6	10^6				
		10^7	104	117	1.2 x 10^9	
		10^8	15	12		
T98G	3616	10^5				
		10^6	35*			
		10^7	12			
T98G	899-6	10^5				
		10^6	171			
		10^7	19			
U87	3616	10^5				
		10^6				
		10^7				
U87	899-6	10^5				
		10^6				
		10^7				
SQ20B	3616	10^4				10^2
		10^5				10^3
		10^6				10^4
SQ 20B	899-6	10^4				10^{-5}
		10^5				10^5
		10^6				10^6

U-87

10/31/94-1

1	2	3	4	5	6	7	8	9

PURPOSE -

- Establish dose response survival curve with U87 cell line
- Establish dose response survival curve with U87 cell line + R3616 @ moi = 10^{-1}

DESIGN -

- Fixed variables - cell type U87 (human glioma)
  - intervals = infect × 2° → subcult → 4° → XRT
- Study variables - virus: None, R3616 @ moi = 10^{-1}
  - XRT dose: 0, 2, 3, 5, 7, 9 Gy

U-87

10/31/94-2

METHODS

① Plate U87 cells on 60 mm dish & grow to ~80% confluence. Need 5 plates. (plate 5×10^5/plate)

11/2/94 ② Count 1 plate & infect 2 plates @ MOI 10^{-1} (R3616) for 2°

1.2×10^6 cells $\times 10^{-1}$ (moi) $= \frac{1.2 \times 10^5 \text{ PFU}}{6 \times 10^5 \text{ PFU/ml dil.}} = .2$ ml + .8 ml

③ After 2° infection, subculture to 100mm plates (in duplicate

RT	Virus	# plated		
0	−	500	1000	5000
2	−	1000	5000	10^4
3	−	5000 ~~10^4~~	10^4	2×10^4
5	−	10^4	2×10^4	
7	−	~~10^4~~	5×10^4	10^5
9	−	5×10^4	10^5	
0	+	1000	2000	5000
2	+	5000	10^4	
3	+	10^4	2×10^4	
5	+	10^4	2×10^4	5×10^4
7	+	5×10^4	10^5	
9	+	5×10^4	10^5	

11/28/94 ④ Stain & count

U-87

10/31/94-3

RESULTS

R3616 moi	RT Dose	# plated	# counted		ave survival	P.E.	rel. survival
0	0	500	0	0	5×10^{-4}	5×10^{-4}	1
		1000	0	0			
		5000	2	3			
	2	1000	0	0	2.7×10^{-4}		.53
		5000	0	1			
		10^4	4	3			
	3	5000	0	0	1.55×10^{-3}		3.1
		10^4	2	6			
		2×10^4	47	38			
	5	2×10^4	13	5	3.2×10^{-4}		.63
		10^4	1	0			
	7	5×10^4	5	5	6×10^{-4}		1.2
		10^5	104	67			
	9	5×10^4	1	2	4.1×10^{-4}		.83
		10^5	69	52			

U-87

10/31/94 - 4

RESULTS (cont.)

R3616 mol	RT Dose	# Plated	# counted		Ave Surv.	P.E.	rel Surv.	
0.1	0	1000	0	0	3×10^{-4}	3×10^{-4}	1	?
		2000	0	0				
		5000	2	1				
	2	5000	0	0	3×10^{-4}		1	
		10^4	3	3				
	3	10^4	3	0	1.1×10^{-3}		3.7	
		2×10^4	41	—				
	5	10^4	2	0	1.4×10^{-3}		4.9	
		2×10^4	20	23				
		5×10^4	105	85				
	7	5×10^4	36	16	8.7×10^{-4}		2.9	
		10^5	92	116				
	9	5×10^4	20	10	2.6×10^{-4}		.88	
		10^5	4	45				

U-87
10/31/94-5

CONCLUSIONS

1. U87 is a fairly radioresistent cell line (surviving cells @ 9 Gy)
2. Does not appear to be a major impact of R3616 on dose response curve.
3. Plating efficiency extremely low ($\approx 5 \times 10^{-4}$) Cannot draw any quantitative conclusions
4. Scit estimates SF_2 (in vitro) at 0.5.
5. Repeat using conditioned media

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 22004

ARC USE ONLY

P.O. # ______

ORDER DATE ______

REF. # ______

CONTACT ______

EST AMT ______

SCHED DEL ______

SPECIAL ROUTING

NON COM VENDOR ______

FLAGGED BY PROTOCOL ______

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: Dr. Greg Sibley DATE: 11/9/94

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: [signature]

FAS ACCOUNT: 6-95150-5100 P.I.: Hallahan

VENDOR: FCRI PROTOCOL: 58671

REQUESTED DELIVERY DATE: 11/17/94 PHONE: 2-6819

SPECIES: mouse QUANTITY: 48

STRAIN: Athymic Nude SEX: M F EITHER

WEIGHT/AGE: 5-6wks ALTERNATE WEIGHT/AGE: ______

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☑ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: ______

HOUSE AT: ___ CARLSON ___ WYLER X CLSC ___ FMI ___ OTHER

11/4/94 - 1

EFFICIENCY LINE® 22-206

PURPOSE -

- Establish dose response survival curve with U87 cell line & 899-6 virus @ 10^{-1} & 10^{-3}

DESIGN -

- Fixed variables - cell type U87
  - intervals = infect × 2° → Subcult → 4° → XRT

- Study variables - virus MOI = 10^{-1} & 10^{-3} 899-6
  - RT dose : 0, 2, 3, 5, 7, 9 Gy

11/4/94 - 2

METHODS —

11/4/94 ① Plate U87 cells on 3 60mm dishes = 5×10^5

11/10/94 ② ~~Count~~ Grow to subconfluence, Count 1 dish to calculate MOI. Count = 3×10^6 cells

New 888-6 titer = 1.2×10^9 PFU/ml

For MOI 10^{-1}: 3×10^5 PFU / 1.2×10^6 PFU/ml dil = ~~.25 ml~~ .25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

For MOI 10^{-3}: 3×10^3 PFU / 1.2×10^4 PFU/ml dil = ~~250 ml~~ .25 ml

.5 ml virus + 1.5 media = 2 ml (add 1 ml to plate)

③ Infect x 2° then subcult to 100mm plates (in dupl.)

RT	Virus	# plated		
0	10^{-1}	1000	2000	5000
2	10^{-1}	5000	10^4	
3	10^{-1}	10^4	2×10^4	
5	10^{-1}	10^4	2×10^4	
7	10^{-1}	5×10^4	10^5	
9	10^{-1}	5×10^4	10^5	
0	10^{-3}	1000	2000	5000
2	10^{-3}	5000	10^4	
3	10^{-3}	10^4	2×10^4	
5	10^{-3}	10^4	2×10^4	
7	10^{-3}	5×10^4	10^5	
9	10^{-3}	5×10^4	10^5	
0	Ø	500	1000	5000

11/28/94 ④ Stain & count (perfect size to count)

U-87
11/4/94-3

Results -

899-6 MGBI ~~LAMS~~	RT	# Plated	# Counted		ave surv.	P.E.	rel surv.
10^{-1}	0	1000	0	—	—	.008	1
		2000	1 —	1 —	—		
		5000	23	57	.008		
	2	5000	11	3	.0045		.56
		10^4	74	47			
	3	10^4	16	10	.0024		.29
		2×10^4	68	TM			
	5	10^4	0	5	.002		.25
		2×10^4	55	24			
	7	5×10^4	61	81	.0014		.18
		10^5	TM	TM			
	9	5×10^4	17	25	.0004		.05
		10^5	84?	TM			
10^{-3}	0	1000	2 -	0 -	.0081 ~~.0064~~	.0081 ~~.0064~~	1
		2000	7 -	9 -			
		5000	43	38			
	2	5000	4 -	3 -	.0058		.71
		10^4	60	55			
	3	10^4	37	50	.0044		.54
		2×10^4	TM	TM			
	5	10^4	10	21	.0026		.32
		2×10^4	63	62			
	7	5×10^4	98	97	.002		.24
		10^5	TM	TM			
	9	5×10^4	34	39	.0007		.09
		10^5	TM	TM			

U87 - TNF

11/9/94 - 1

Purpose - Quantify TNF production in U87 cell line with 899-6 virus +/- 5 Gy RT. Use 3616 virus as control

Design -

- Fixed variables - cell type U87
  - intervals = infect x 2 hrs → RT
  - aliquot media @ 0, 2, 4, 6 hrs then subculture & save 10^6 cells
- Study variables - virus: None, 3616, 899-6 @ MOI = 10
  - RT: 0 Gy, 5 Gy

Conclusion - 10^5 PFU too little & slow TNF production c̄ 899-6

11/9/94-2

METHODS -

11/4/94 (1) Plate U87 cells in 60 mm dishes.
Grow to subconfluent. Need ~~20~~ 15 dishes

11/13/94 (2) ~~Count 1 dish to determine # PFU needed for MOI of 10^{-1}.~~ ← No. Infect $\bar{c}$ 10^5 PFU (use this also for other cell lines to compare TNF production i.e. TNF production should be dependent only on PFU & not on cell number & (MOI not important)).

899-6 titer = 1.2×10^9 PFU/ml : $\frac{10^5 \text{ PFU}}{1.2 \times 10^5 \text{ PFU/ml}}$ = 0.83 ml of 1.2×10^5 dil (+ .17 media)

3616 titer = 2.3×10^9 PFU/ml : $\frac{10^5}{2.3 \times 10^5 \text{ PFU/ml}}$ = 0.43 ml " 2.3×10^5 " (+ .57 media)

(3) Infect cells x 2° in 1 ml 199V media.
~~Add~~

(4) Remove innoculum & add 6 ml of 10% FCS media (U87 media) + IgG.

(5) Irradiate $\bar{c}$ 5 Gy

(6) Aliquot 1 ml media @ 0, 2, 4, 6° & subculture in 5 ml ~~@ 10^6 cells/ml~~ → save ~~as~~ 1 ml as "pellet".

(7) Centrifuge aliquots before using for TNF ELISA assay

(8) To calculate total TNF:

899-6 = ([0°] x 1ml) + ([2°] x 1ml) + ([4°] x 1ml) + ([6°] x 3ml) + ([6° P] x 2 ...

3616 = ([6°] x 6 ml) + ([6° P] x 2 ml)

Ø = ([6°] x 6 ml) + ([6° P] x 2 ml)

V-87 R899-6

11/16/94 - 1

Purpose - Generate Cell Survival Curve for U-87 cells with different MOI's of 899-6 virus.

Design - Fixed variables - cell type U87
- intervals = infect x 2°
- virus = 899-6

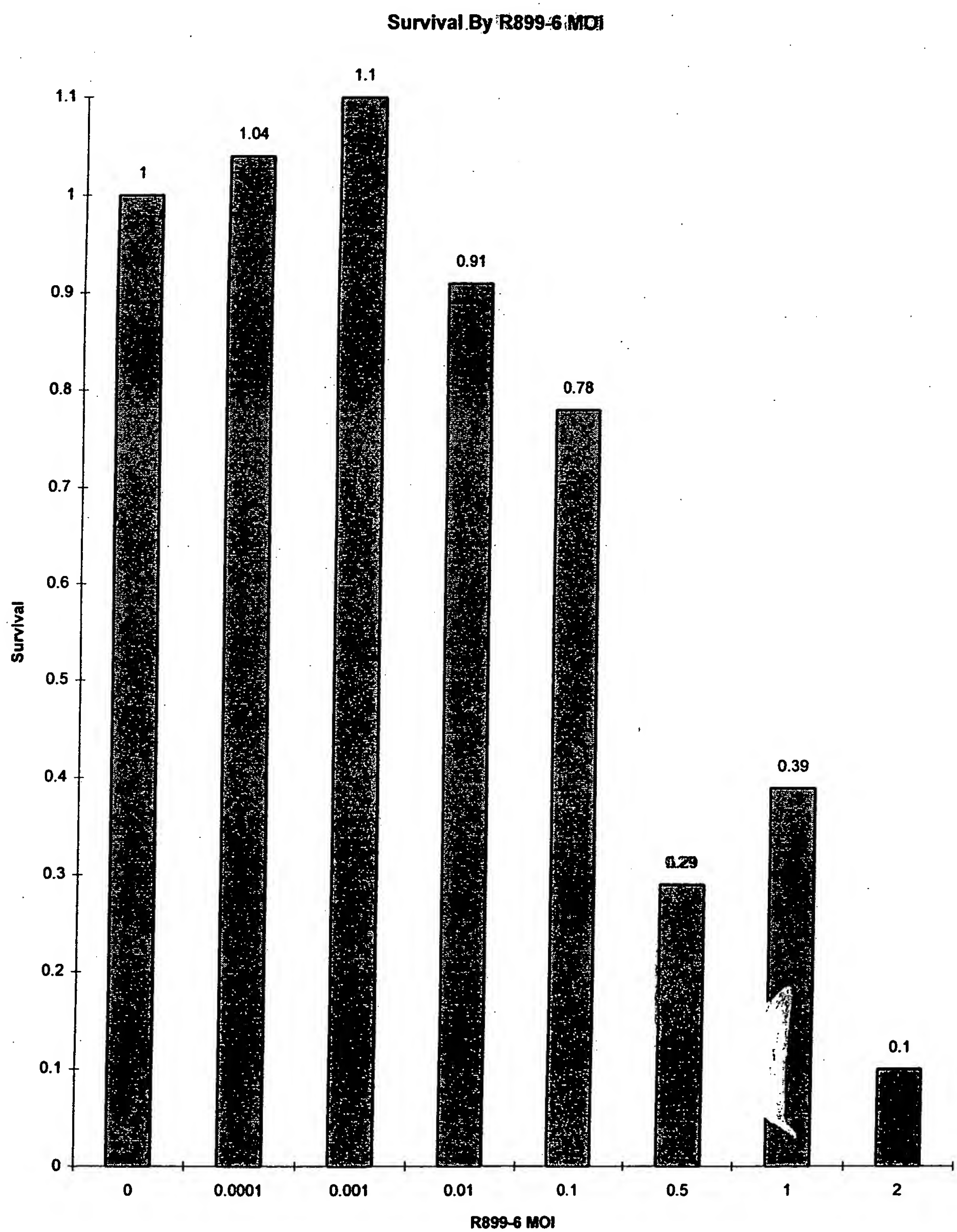
Study variables = MOI = 2, 1, .5, 10^{-1}, ~~5×10^{-2}~~, 10^{-2}, ~~5×10^{-3}~~, 10^{-3}, 10^{-4}

V-87

11/16/94 - 3

RESULTS

899-6 moi	# Plated	# counted		ave. survival	P.E.	rel. survival
0	500	12	—	.021	.021	1
	1000	25	–			
	2000	35	45			
10^{-4}	1000	24	30	.022	"	1.04
	2000	35	44			
	5000	Tm	Tm		"	
10^{-3}	2000	70	62	.023	"	1.10
	5000	104	91			
	10^4	Tm				
10^{-2}	2000	24	41	.019	"	.91
	5000	95	112			
	10^4	Tm	Tm			
10^{-1}	2000	32	34	.016	"	.78
	5000	Tm	Tm			
	10^4	Tm				
0.5	5000	34	28	.0062		.29
	10^4	33	Tm			
	10^5	Tm				
1	10^4	46	36	.0082		.39
	10^5	Tm	Tm			
	10^6	Tm				
2	10^5	>150 Tm	>150 Tm	>.0015		>.07
	10^6	Tm				


Survival By R899-6 MOI
1.1
1.04
1
0.91
0.78
0.39
0.29
0.1
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Survival
0
0.0001
0.001
0.01
0.1
0.5
1
2
R899-6 MOI

V-87

11/16/94 -4

Conclusions -

1. R899-6 doesn't appear to have an appreciable effect until moi ~ 0.1
2. ? TCD_{50} ~ moi = 0.5
3. Repeat using moi's of 0, 10^{-2}, 10^{-1}, 0.5, 1, 2, 3, 5.
4. Plating efficiency still very low (2%), try using conditioned media (50/50)

EFFICIENCY LINE 22-206

TNF ELISA   Adeno-TNF Expression (#53)   2.2x10^8 pfu/tu[mor]

	1	2	3 Adeno Egr TNF	4	5 + single 5 Gy fraction	6	7	8	9 Ø XRT	10
A	0		1394 R		1406 R		1410 R		1394 L	
B	15.7		1396 R		1403 R		1415 R		1396 L	
C	31.3		1434 R		1404 R		1412 R		1434 L	
D	62.5		1397 R		1405 R		1413 R		1397 L	
E	125		1398 R		1402 R		1414 R		1398 L	
F	250		1399 R		1407 R		1411 R		1399 L	
G	500		1400 R		15 1408 R		23 1416 R		1400 L	
H	1000		1401 R		1409 R		RIPA BLK		1401 L	

Adeno Ø XRT   5 Gy Adeno-βgal control Ø XRT   2x10^8 pfu/tumor

	11	12	13	14	15	16	17	18	19	20
A	1406 L		1410 L		4 1421		1417		1433 R	
B	1403 L		1415 L		1422		1418		1433 L	untreated tumor
C	1404 L		1412 L		1423		1420		1419	
D	1405 L		1413 L		1425		1424		Medis blk - Gne	
E	1402 L		1414 L		1436 R		1427		1 899-6 0°	
F	1407 L		1411 L		1436 L	untreated tumor	1429		2 " 0 Gy	
38 G	1408 L	46	1416 L		1428		1426		3 899-6 2°	
H	1409 L		RIPA BLK		1435		1430		4 " 0 Gy	

	21	22	23	24	25	26	27	28
A	5 899-6	4°	13 ~~None~~ 899-6	~~P~~	21 None	6°	29 3616	6°
B	6 "	0 Gy	14 "	5 Gy	22 "	0 Gy	30 "	0 Gy
C	7 899-6	6°	15 899-6	4°	23 None	P	31 3616	P
D	8 "	0 Gy	16 "	5 Gy	24 "	0 Gy	32 "	0 Gy
E	9 899-6	P 0 Gy	17 899-6	6°	25 None	6°	33 3616	6°
F	10 "	"	18 "	5 Gy	26 "	5 Gy	34 "	"
G	11 ~~None~~ 899-6	0°	19 899-6	P	27 None	P	35 3616	6°
H	12 "	5 Gy "	20 "	5 Gy	28 "	5 Gy	36 "	5 Gy

[TNF] By Virus Type (10 exp5 PFU) and RT Dose in U-87 Cells

Virus	Dose	Time	[TNF]	Total [TNF]
899-6	0	0	0.19	62.52
899-6	0	2	2.34	
899-6	0	4	4.99	
899-6	0	6	11.04	
899-6	0	p	10.94	
899-6	5	0	0.94	64.37
899-6	5	2	1.49	
899-6	5	4	4.99	
899-6	5	6	12.29	
899-6	5	p	10.04	
None	0	6	6.64	42.62
None	0	p	1.39	
None	5	6	3.64	24.42
None	5	p	1.29	
R3616	0	6	5.44	42.92
R3616	0	p	5.14	
R3616	5	6	11.09	
R3616	5	p	5.34	

MOLECULAR DEVICES V_{max} KINETICS MICROPLATE READER PLATE #: 1

ASSAY : ______________________ DATE: ___/___/___

OPERATOR: ______________________ **O.D. LIMIT:** 3.000 TIME: ___:___ AM/PM

WAVELENGTH: 450nm - OPT 2 **READ MODE:** OPTICAL DENSITY **AUTO MIX:** ON

NOTES: ______________________ **CAL:** ON

OPTICAL DENSITY

	25	26	27	28	29	30	31	32	33	34	35	36
A	0.063	0.176	0.073	0.066	-0.000	0.000	0.000	-0.000	-0.000	-0.000	-0.000	0.000
B	0.081	0.070	0.095	0.132	-0.000	-0.000	-0.000	0.000	-0.000	-0.000	-0.000	-0.000
C	0.067	0.076	0.080	0.077	-0.000	-0.000	0.000	-0.000	-0.000	-0.000	-0.000	-0.000
D	0.068	0.074	0.090	0.113	-0.000	-0.000	-0.000	0.000	-0.000	-0.000	-0.000	-0.000
E	0.073	0.079	0.095	0.120	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	-0.000
F	0.101	0.077	0.106	0.158	-0.000	-0.000	-0.000	-0.000	-0.000	0.000	-0.000	-0.000
G	0.076	0.068	0.096	0.094	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000
[illegible]	[illegible].069	0.070	0.091	0.083	-0.001	-0.001	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000

$(y - .0643)/.005$

$y = .005x + .0643$

$y = .0657e^{.6604x}$

$\frac{\ln y - \ln .0657}{.6604} = \cancel{.6004}\,x$

MOLECULAR DEVICES — Vmax KINETIC MICROPLATE READER — PLATE #: 1

ASSAY : V87 — DATE: 11/17/94

OPERATOR: Greg — O.D. LIMIT: 3.000 — TIME: ___:___ AM/PM

WAVELENGTH: 450nm - OPT 2 — READ MODE: OPTICAL DENSITY — AUTO MIX: OFF

NOTES: — CAL: ON

Standards

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.071	0.086	0.097	0.101	0.092	0.093	0.080	0.073	0.090	0.072	0.127	0.101
B	0.123	0.144	0.070	0.072	0.375	0.366	0.272	0.236	*	*	[illegible]	0.091
C	0.199	0.198	*	*	[illegible]	[illegible]	0.490	[illegible]	1.311	1.202	0.366	[illegible]
D	0.371	0.367	0.343	0.342	0.088	0.088	0.161	0.149	1.578	1.442	0.363	[illegible]
E	0.670	0.727	0.154	0.153	0.297	0.288	*	*	1.205	1.191	0.127	[illegible]
F	1.333	1.385	0.660	0.680	0.647	0.635	[illegible]	0.110	2.623	2.650	0.198	0.166
G	2.807	2.860	*	*	1.779	1.877	0.129	[illegible]	0.728	0.700	0.121	0.116
H	*	*	0.203	0.193	2.161	2.483	0.086	0.063	0.131	0.039	0.600	[illegible]

Lisa & Helene's Stuff

MOLECULAR DEVICES — Vmax KINETIC MICROPLATE READER — PLATE #: 1

ASSAY : — DATE: ___/___

OPERATOR: — O.D. LIMIT: 3.000 — TIME: ___:___ AM/PM

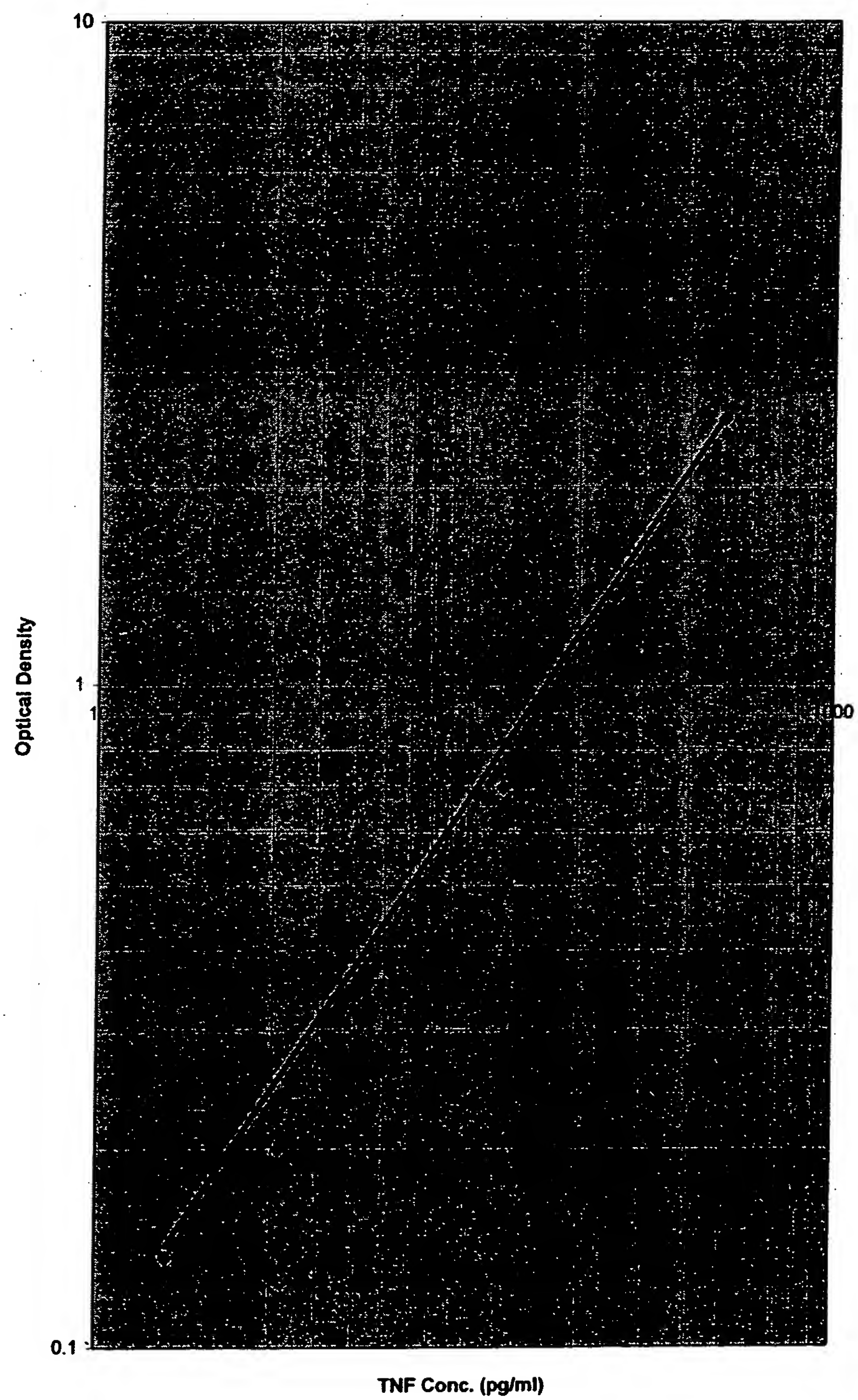
WAVELENGTH: 450nm - OPT 2 — READ MODE: OPTICAL DENSITY — AUTO MIX: OFF

NOTES: — CAL: ON

OPTICAL DENSITY

	13	14	15	16	17	18	19	20	21	22	23	24
A	0.075	0.070	0.149	0.188	0.327	0.324	[illegible]	0.152	0.097	0.087	0.077	0.070
B	0.225	0.267	0.148	0.180	0.137	0.151	0.106	0.109	0.090	0.083	0.072	0.068
C	0.810	0.844	0.478	0.609	0.074	0.075	0.219	0.211	0.127	0.117	0.088	0.092
D	0.250	0.263	0.348	0.235	0.128	0.125	0.064	0.069	0.114	0.120	0.086	0.091
E	0.583	0.716	0.079	0.082	0.083	0.097	0.055	0.080	0.109	0.122	0.120	0.125
F	0.126	0.134	0.123	0.183	0.048	0.048	0.069	0.078	0.124	0.121	0.130	0.128
G	0.271	0.242	0.058	0.102	0.059	0.062	0.065	0.073	0.067	0.071	0.109	0.119

TNF ELISA Plot 11/17/94



V-87
R899-6
11/29/94-1

1	2	3	4	5	6	7	8	9

PURPOSE – Generate cell survival curve for V-87 cells with different moi's of 899-6 virus

Design – Fixed variables – cell type V87
– intervals = infect x 2°
– virus 899-6

Study variables – moi: 0, 10^{-2}, 10^{-1}, 0.5, 1, 2, 3, 5

V-87

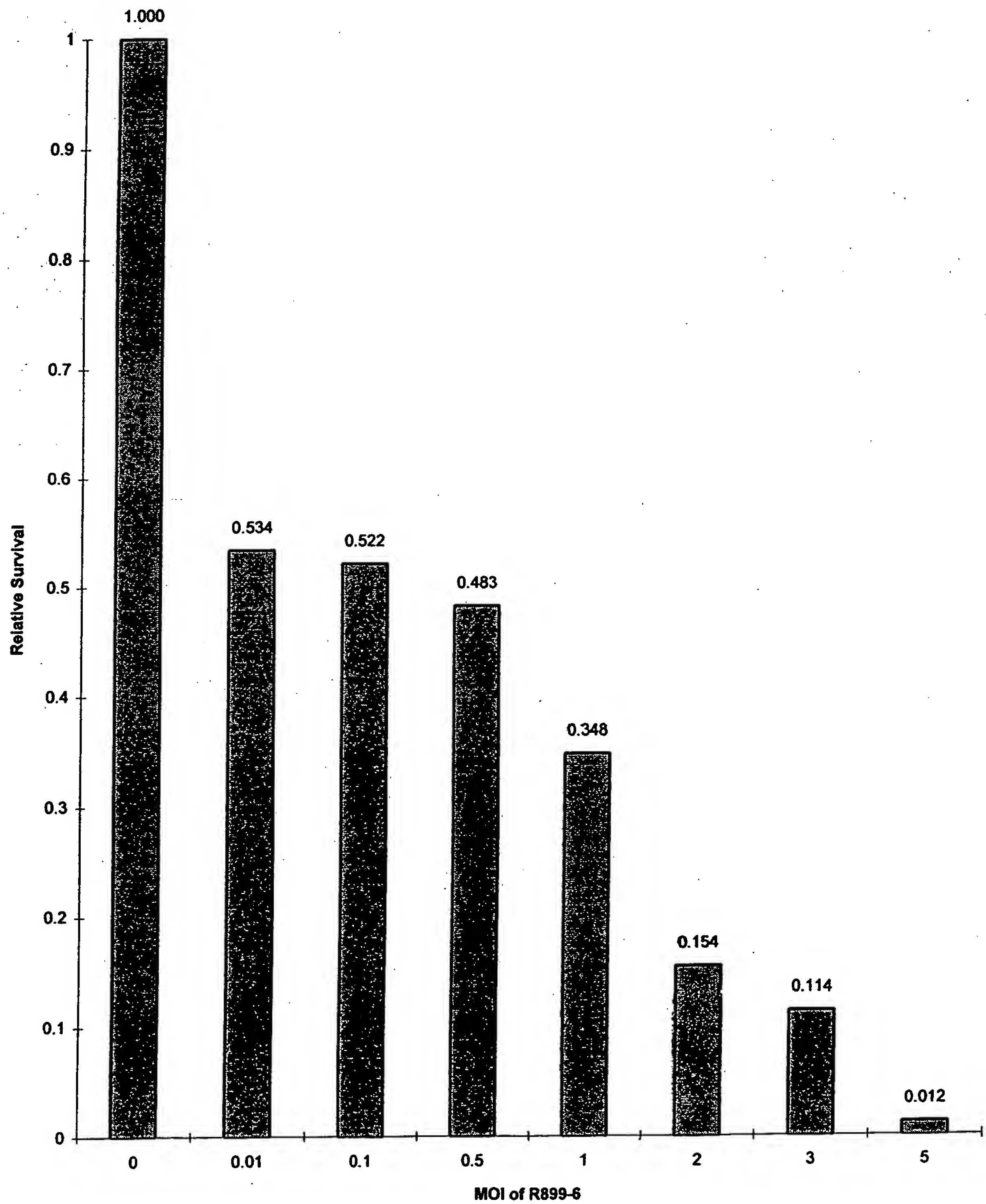
11/29/94 - 3

Results

899-6 MOI	# Plated	# Counted			% Surv.	P.E.	Rel Surv
0	2000	21	12	19			
	5000	68	64	59			
10^{-2}	2000	5	6				
	5000	53	28	48			
10^{-1}	2000	4	11	6			
	5000	30	50	35			
0.5	5000	34	22	21			
	10^4	51	70	35			
1	10^4	36	37	24			
	2×10^4	90	83	80			
2	10^4	18	13	17			
	2×10^4	33	25	44			
3	2×10^4	18	17	14			
	5×10^4	69	84	89			
5	5×10^4	7	8	5			
	10^5						

11/29/94: Survival of U-87 Cells by MOI of R899-6							
MOI	# plated	# counted			Ave. Surv.	P.E.	Rel. Surv.
0	2000	21	12	19	0.0107	0.0107	1
	5000	68	64	59			
0.01	2000	5	6	6	0.005717	0.0107	0.534268
	5000	53	28	48			
0.1	2000	4	11	6	0.005583	0.0107	0.521807
	5000	30	50	35			
0.5	5000	34	22	21	0.005167	0.0107	0.482866
	10000	51	70	35			
1	10000	36	37	24	0.003725	0.0107	0.348131
	20000	90	83	80			
2	10000	18	13	17	0.00165	0.0107	0.154206
	20000	33	25	44			
3	20000	18	17	14	0.001215	0.0107	0.113551
	50000	69	84	89			
5	50000	7	8	5	0.000133	0.0107	0.012461
MOI	Rel. Surv.						
0	1.000						
0.01	0.534						
0.1	0.522						
0.5	0.483						
1	0.348						
2	0.154						
3	0.114						
5	0.012						

Relative Survival By R899-6 MOI in U-87 Cells



U-87 Mycoplasma Assay

12/6/94

PURPOSE - Rule out mycoplasma infection as reason for poor plating efficiency c̄ U87 cells

METHODS - See attached protocol

CONCLUSION - No mycoplasma infection

U-87 - TNF

12/7/94 - 1

Purpose - Quantify TNF production in U87 cell line c̄ R3616, 899-6 & Ø virus c̄ 0 or 9 Gy ~~(moi of virus = 0.5)~~ Use 2×10^6 PFU (~moi = 1)

METHODS -

1. Plate U87 cells in 60 mm dishes & grow to subconfluence
2. 899-6: 2×10^6 PFU / 1.2×10^7 PFU/ml = .17 ml
   3616: 2×10^6 PFU / 2.3×10^6 PFU/ml = .87 ml
3. Infect × 2° in total volume 1 cc 199V media
3. Remove inoculum & add 6 ml of 10% FCS media
~~4. Aliquot 1 ml media @ 2, 6~~
4. Irradiate c̄ 9 Gy RT 11am 5pm 9pm 9am 3pm
5. Aliquot 1 ml media @ 2°, 6°, 12°, 24°, 30°
   then subculture in 3ml and save 1 ml as pellet

TNF ELISA Assay: U-87 Cells Infected with R899-6, R3616, or No Virus (MOI=0.5) +/- 9 Gy RT (12/7/94)

Standards

reading 1	reading 2	[TNF]	ave. reading
0.087	0.087	15.6	0.087
0.119	0.137	31.3	0.128
0.205	0.217	62.5	0.211
0.39	0.407	125	0.399
0.733	0.756	250	0.745
1.354	1.459	500	1.407
1.997	2.284	1000	2.141

				Virus	Dose	Time	Total [TNF]
0.044	0.044	7.8	0.044	None	0	0	62.60
0.045	0.046	8.1	0.046	None	0	10	
0.04	0.046	7.6	0.043	None	0	21	
0.044	0.042	7.6	0.043	None	0	27	
0.043	0.047	8.0	0.045	None	0	P	
0.04	0.048	7.8	0.044	None	9	0	67.73
0.051	0.046	8.8	0.049	None	9	10	
0.052	0.054	9.8	0.053	None	9	21	
0.051	0.048	9.0	0.050	None	9	27	
0.043	0.045	7.8	0.044	None	9	P	
0.047	0.041	7.8	0.044	R3616	0	0	72.24
0.047	0.044	8.1	0.046	R3616	0	10	
0.042	0.055	8.8	0.049	R3616	0	21	
0.054	0.059	10.6	0.057	R3616	0	27	
0.05	0.047	8.8	0.049	R3616	0	P	
0.046	0.042	7.8	0.044	R3616	9	0	67.48
0.046	0.04	7.6	0.043	R3616	9	10	
0.051	0.05	9.2	0.051	R3616	9	21	
0.046	0.048	8.4	0.047	R3616	9	27	
0.048	0.048	8.7	0.048	R3616	9	P	
0.054	0.05	9.6	0.052	R899-6	0	0	3004.23
1.476	1.587	633.2	1.532	R899-6	0	10	
1.836	1.48	698.6	1.658	R899-6	0	21	
1.752	1.973	806.9	1.863	R899-6	0	27	
0.08	0.08	16.3	0.080	R899-6	0	P	
0.047	0.051	8.9	0.049	R899-6	9	0	3323.99
0.939	*0.829*	*320.5*	*0.884*	*R899-6*	*9*	*6*	
1.503	1.569	635.5	1.536	R899-6	9	10	
1.83	1.96	824.4	1.895	R899-6	9	21	
2.076	1.99	899.4	2.033	R899-6	9	27	
0.09	0.089	18.8	0.090	R899-6	9	P	

U-87 ~~TNF~~
R3616
12/12/94

Purpose – Survival of U87 cells c̄ ~~[illegible]~~ R3616 @ MOI of 0 (control) & 0.5 c̄ RT ~~doses~~: 0, 3, 5, 7, 9 Gy

METHODS –

① Plate 10^6 cells in 60 mm dish (12/9) & grow to confluence

② Count a dish & infect ~~[illegible]~~ one dish c̄ MOI 0.5 of ~~[illegible]~~ R3616

12/13/94 ③ Infect x 2° in 1 ml 199V @ 37°

④ Subculture into 100 mm plates c̄ 50% Cond. Media (+ IgG). Wait 6°.

⑤ Irradiate @ 0, ~~1~~ 3, 5, 7, 9 Gy

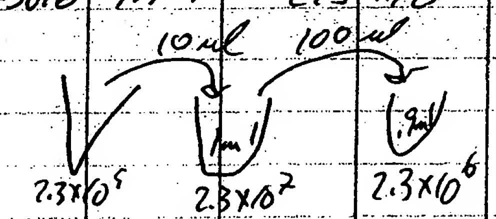
⑥ Incubate x 3 wks

⑦ Stain & Count

~~⑧ Aliquot media [illegible] @ [illegible] for [TNF]~~
~~(?, is there induction c̄ ↑ doses RT~~

Dish count = 2.3×10^6 cells

R3616 titer = 2.3×10^9 PFU/ml



Use 0.5 cc 2.3×10^6 dilution = MOI 0.5

V87
12/12/94

Results –

Vins	RT	#plated	#counted		ave surviving	P.E.	rel survival
0	0	2000	13	7			
		5000	40	35			
	3	5000	0	0			
		10^4	12	18			
	5	10^4	8	8			
		2×10^4	28	46			
	7	10^4	5	1			
		2×10^4	6	9			
	9	2×10^4	4	1			
		5×10^4	3	0			
0.5	0	5000	3	2			
		10^4	5	7			
	3	10^4	2	0			
		2×10^4	4	2			
	5	2×10^4	1	0			
		5×10^4	4	8			
	7	2×10^4	1	0			
		5×10^4	5	2			
	9	5×10^4	0	2			
		10^5	0	5			

V-87
R899-6
12/18/94-1

Purpose - Generate cell survival curve for V-87 cells with RT doses 0, 5, 9 Gy & MOI's of 899-6 = ~~1~~ 0.5, ~~0.1~~ 0, 0

METHODS ① Plate 10^6 cells per 60 mm dish = V-87
② When subconfluent (2 days) infect c̄ virus in 1cc 199V × 2°.

~~MOI~~ Titer R899-6 $= 1.2 \times 10^9$ pfu/ml

10 ml → 1 ml, 5 ml, 4.5
1.2×10^9 1.2×10^7 1.2×10^6

MOI = 1 = ~~1.2×10^6~~ $1.6 \times 10^6 / 1.2 \times 10^7 = 0.133$

MOI = 0.5 = $0.8 \times 10^6 / 1.2 \times 10^6 = .667$

~~MOI = 0.1 = $1.6 \times 10^5 / 1.2 \times 10^6 = .133$~~

③ Subculture into 10 mm dishes at appropriate concentration c̄ 50% Conditioned media.
④ Let cells adhere × 6°
⑤ Irradiate
⑥ Incubate × 3 wks, Stain & count.

U-87

12/18/94-2

RESULTS –

899-6 moi	RT dose	# plated	# counted		ave. survival	P.E.	rel survival
0	0	2000	80	100			
		5000	159	165			
	5	10^4	32	29			
		2×10^4	72	95			
	9	2×10^4	7	6			
		5×10^4	12	8			
0.1	0	2000	40	58			
		5000	81	93			
	5	10^4	14	18			
		2×10^4	46	43			
	9	2×10^4	1	2			
		5×10^4	0	–			
0.5	0	5000	71	73			
		10^4	104	117			
	5	2×10^4	39	50			
		5×10^4	–	–			
	9	5×10^4	0	1			
		10^5	4	3			
~~1~~	~~0~~	~~10^4~~					
		~~2×10^4~~					
	~~5~~	~~2×10^4~~					
		~~5×10^4~~					
	~~9~~	~~5×10^4~~					
		~~10^5~~					

12/20/94

PURPOSE – Assess toxicity of R899-6 & R3616 in nude mice

METHODS –

① 8 mice

R899-6: 10 ul of 1.2×10^9 PFU/ml = 1.2×10^7 PFU (2 mice)
10 ul of 1.2×10^8 PFU/ml = 1.2×10^6 PFU (2 mice)

R3616: 10 ul of 2.3×10^9 PFU/ml = 2.3×10^7 PFU (2 mice)
10 ul of 2.3×10^8 PFU/ul = 2.3×10^6 PFU (2 mice)

Cage AA104551 –	1681	Ø	R899-6	1.2×10^7
	1682	L	"	"
	1683	R	"	1.2×10^6
	1684	LR	"	"
Cage AA104552 –	1685	Ø	R3616	2.3×10^7
	1686	L	"	"
	1687	R	"	2.3×10^6
	1688	LR	"	"

② Injected 3:00 pm 1/5/94

RESULTS –

No local or systemic effects noted
Mice examined twice weekly for weeks
Sac'd on

U-87 – Plating efficiency

1/9/95

PURPOSE – To improve plating efficiency of U87 cell line by trying different media

METHODS –

Base media	% FCS	CM	# plated	# counted	
MEM/Napyr/aa	10%	+	1000	infected	infected
"	10	+	5000	infected	infected
"	15	+	1000	159	infected
"	15	+	5000	infected	infected
"	20	+	1000	infected	infected
"	20	+	5000	tm	infected
DME-F12	10%	+	1000	infected	infected
"	10	+	5000	tm	tm
"	15	+	1000	91	infected
"	15	+	5000	tm	tm
"	20	+	1000	131	50
"	20	+	5000	tm	infected

January 10, 1995

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

As per our telephone conversation on 1/10/95, please place the following mice on TMP-SMZ (Bactrim) for 1 week:

> Cages AA105283, AA104542 through AA104545, AA104547 through AA10450, and AA107678 through AA107710.

This should include **all** mice from Hallahan's Lab (Radiation Oncology) currently in Cummings Room #1053.

Thank you. If you have any questions you can contact me at beeper #3439 or contact Helena at 2-0294.

Sincerely,

Gregory S. Sibley, M.D.

THE UNIVERSITY OF CHICAGO ANIMAL RESOURCES CENTER **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 18777

ARC USE ONLY

P.O. #

ORDER DATE

REF. #

CONTACT

EST AMT

SCHED DEL

SPECIAL ROUTING

NON COM VENDOR

FLAGGED BY PROTOCOL

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ______ SHIPMENTS ON A ______ BASIS

REQUEST BY: GREGORY SIBLEY, MD DATE: 1/16/95

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: Malcolm R. [illegible]

2-73731-5100

FAS ACCOUNT: [illegible]

VENDOR: FCR I

REQUESTED DELIVERY DATE: 1/23/95

P.I.: Hallahan

PROTOCOL: 58671

PHONE: ~~2-6819~~ 2-0294

SPECIES: Mouse

QUANTITY: 60

STRAIN: Athymic Nude

SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE:

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS:

HOUSE AT: ____ CARLSON ____ WYLER X CLSC ____ FMI ____ OTHER

U87 TNF (intervals)

1/17/95

PURPOSE - To determine optimal interval from infex (R899-6) to RT (9 Gy) to optimize TNF production in U87 cells

METHODS -

1/16/95 ① Plate 10^6 U87 cells per 60 mm dishes. Incubate ~ 36° for ~ 2×10^6 cells

② Infect plates with 10^6 PFU of R899-6 at

1/17/95 12° prior to RT = 10 pm

1/18/95 8° prior to RT = 2 Am

4° " " " = 6 Am (Actually 6^{45} = 3.25 hrs)

2° " " " = 8 Am

Titer R899-6 = 1.2×10^9 PFU/ml

∴ Use 0.83 ml of 1.2×10^6 PFU/ml dilution + 199U media to total vol = 1.5 cc

③ Add 2.5 ml 10% FCS media ⊕ IgG (total vol 4 cc)

"Control" dishes ⇒ Aliquot 1 ml as "supernatant"

~~Ey~~ Decant remaining media

Trypsinize cells c̄ 1 ml trypsin + 1 ml Verse

then add 2 ml media (total vol = 4.

Aliquot 1 ml as "pellet"

④ For "treatment" dishes RT c̄ 9 G.

Wait 7° then repeat aliquot p. edure for supernatant & pellet as above

TNF ELISA 1/20/95 U-87 Cells

reading 1	reading 2	average	[TNF]	Time	Sample	Average	Total [TNF]	Rel. [TNF]
1.267	1.308	1.2875	325.978	12 hours	control-super	1283.22	1623.47	1239.04
1.248	1.256	1.252	315.632	12 hours	control-super			
0.38	0.353	0.3665	76.5162	12 hours	control-pellet	340.25		
0.474	0.399	0.4365	93.6086	12 hours	control-pellet			
2.331	2.31	2.3205	643.123	12 hours	9 Gy-super	2394.87	2862.506	
2.053	2.027	2.04	554.311	12 hours	9 Gy-super			
0.505	0.534	0.5195	114.425	12 hours	9 Gy-pellet	467.638		
0.537	0.541	0.539	119.394	12 hours	9 Gy-pellet			
0.417	0.422	0.4195	89.416	8 hours	control-super	360.864	642.5502	2071.4
0.421	0.431	0.426	91.016	8 hours	control-super			
0.311	0.34	0.3255	66.73	8 hours	control-pellet	281.686		
0.343	0.37	0.3565	74.113	8 hours	control-pellet			
1.455	1.503	1.479	382.519	8 hours	9 Gy-super	1953.96	2713.952	
2.127	2.208	2.1675	594.462	8 hours	9 Gy-super			
0.587	0.607	0.597	134.332	8 hours	9 Gy-pellet	759.989		
0.957	1.058	1.0075	245.662	8 hours	9 Gy-pellet			
0.065	0.058	0.0615	9.76247	3.25 hours	control-super	40.8921	130.1639	1371.14
0.065	0.068	0.0665	10.6836	3.25 hours	control-super			
0.085	0.087	0.086	14.3726	3.25 hours	control-pellet	89.2718		
0.158	0.17	0.164	30.2632	3.25 hours	control-pellet			
0.933	0.991	0.962	232.909	3.25 hours	9 Gy-super	906.04	1501.304	
0.901	0.931	0.916	220.111	3.25 hours	9 Gy-super			
0.709	0.714	0.7115	164.467	3.25 hours	9 Gy-pellet	595.264		
0.599	0.586	0.5925	133.165	3.25 hours	9 Gy-pellet			
0.059	0.068	0.0635	10.1296	2 hours	control-super	41.0714	131.0586	2360.16
0.065	0.065	0.065	10.4061	2 hours	control-super			
0.067	0.112	0.0895	15.0494	2 hours	control-pellet	89.9873		
0.179	0.146	0.1625	29.9442	2 hours	control-pellet			
1.414	1.524	1.469	379.538	2 hours	9 Gy-super	1679.41	2491.216	
1.687	1.785	1.736	460.167	2 hours	9 Gy-super			
0.859	0.824	0.8415	199.593	2 hours	9 Gy-pellet	811.806		
0.866	0.866	0.866	206.311	2 hours	9 Gy-pellet			

U-87 TNF (intervals)

1/23/95

PURPOSE - To determine optimal interval from infect (R899-6) to RT (9Gy) to optimize ~~TNF production~~ subsequent TNF induction experiments

METHODS -

1/23/95 ① Plate 10^6 U87 cells per 60 mm dish. Incubate ~~18~~ ~36° for ~2×10^6 cells

② Infect plates $\bar{c}$ 10^6 PFU R899-6
= 0.83 ml of 1.2×10^6 PFU/ml dilution
+ 199V media to Σ 1.5 ml

Infect @ 7^{30} AM

③ Aliquot & trypsinize "control dishes" @
4° $\bar{p}$ infection 11^{30}
6° " " 1^{30}
8° " " 3^{30}

④ Irradiate @ same time points $\bar{c}$ 9 Gy

⑤ Aliquot & trypsinize "treatment dishes" @
7° $\bar{p}$ RT = 6^{30} PM, 8^{30} PM, 10^{30} PM

In Vivo - TNF induction - intervals
~~U87 hind limbs nude mice~~

1/24/95

Purpose - To determine optimal interval from infection → RT in U87 tumors in hind limbs of nude mice for TNF production

Methods -

① Transplant 1mm^3 U87 tumors into hind limbs of nude mice. Grow to ~ 6 mm (range 4.5-8 mm) greatest dimension.

② Infect mice in groups of 4 @ 48°, 24°, 10° & 5° prior to RT.

- 48° = 4^{pm} 1/24
- 24° = 4^{pm} 1/25
- 10° = 7^{Am} 1/26
- 5° = 12^{N} 1/26

0° = 5^{pm} 1/26 = Irradiate c 20 Gy single fraction

③ Sacrifice mice @ 4^{pm}

1/24/95

Greg's Mouse Log

								Tumor size	
	Mouse #	Cage #	DOB	Tumor Date	Infect Date	RT Date	Date Sacrif	1/23/95	1/24
24°	1703	~~AA104542~~	10/10/94	1/10/95	R			7.5	8x5x3.5
	1711	~~AA104543~~	10/10/94	1/10/95	∅ OK			6	5.5x5x2.5
	1712	~~AA104542~~	10/10/94	1/10/95	L			8	10x8.5x6
	1714	~~AA104542~~	10/10/94	1/10/95	LR			6	6x6x3.5
8°	1701	~~AA104543~~	10/10/94	1/10/95	∅			6.5	7.5x6x4
	1715	~~AA104543~~	10/10/94	1/10/95	∅ → L			4.5	5.5x5x3
	1718	~~AA104543~~	10/10/94	1/10/95	LR			5.5	4x5.5x2.5
	1721	~~AA104543~~	10/10/94	1/10/95	∅ → R			7	6x7.5x3
	1716	AA104544	10/10/94	1/10/95				3.5	
	1722	AA104544	10/10/94	1/10/95				3.5	
	1723	AA104544	10/10/94	1/10/95				3	
	1724	AA104544	10/10/94	1/10/95				2	
	~~1689~~	~~AA104546~~	~~10/10/94~~	~~12/6/94~~			1/10/95		
	~~1690~~	~~AA104546~~	~~10/10/94~~	~~12/6/94~~			1/10/95		
	~~1691~~	~~AA104546~~	~~10/10/94~~	~~12/6/94~~			1/10/95		
	~~1692~~	~~AA104546~~	~~10/10/94~~	~~12/6/94~~			1/10/95		
	1693	AA104547	10/10/94	12/6/94	∅			10	
	~~1694~~	~~AA104547~~	~~10/10/94~~	~~12/6/94~~			1/24/95	20	
	1695	AA104547	10/10/94	12/6/94	Sac			0	
48°	1696	~~AA104548~~	10/10/94	12/6/94	LR			6	6.5x5.5x3.5
	1698	~~AA104548~~	10/10/94	1/10/95	L			4	9x9.5x8
	1699	~~AA104548~~	10/10/94	1/10/95	R			5	6x5x3
	1700	~~AA104548~~	10/10/94	1/10/95	LR → LRR			6	4.5x2.5x2
	1697	AA104549	10/10/94	1/10/95				3.5	
	1702	AA104549	10/10/94	1/10/95				2	
	1713	AA104549	10/10/94	1/10/95				4.5	
	1717	AA104549	10/10/94	1/10/95				2.5	
	1704	AA104550	10/10/94	1/10/95				3.5	
	~~1705~~	~~AA104550~~	~~10/10/94~~	~~1/10/95~~			~~1/10/95~~		
	1706	AA104550	10/10/94	1/10/95	Sac		1/24/95	0	
	1719	AA104550	10/10/94	1/10/95				3.5	
	1720	AA104550	10/10/94	1/10/95				3.5	
	1681	AA104551	10/10/94	n/a	1/5/95				
	1682	AA104551	10/10/94	n/a	1/5/95				
	1683	AA104551	10/10/94	n/a	1/5/95				
	1684	AA104551	10/10/94	n/a	1/5/95				
	1685	AA104552	10/10/94	n/a	1/5/95				
	1686	AA104552	10/10/94	n/a	1/5/95				
	1687	AA104552	10/10/94	n/a	1/5/95				
	1688	AA104552	10/10/94	n/a	1/5/95				
	1707	AA105283	10/10/94	1/10/95				0	
	1708	AA105283	10/10/94	1/10/95				3	
	1709	AA105283	10/10/94	1/10/95				2	
	1710	AA105283	10/10/94	1/10/95				3	

3/6 8970 control

				wt
1867	23	20.5	12	25.1
sacrificed 1868	23	24	20.5	22.3
1869	21	23	15	24.2
1870	20	18	13	26.9

4550 Control

sacrificed 1719	20	14.5	10	21.8
1722	27	23	19	25.9
1717	17	15.5	12	23.7

5283 891-6

1710	10.5	11.5	3	20.7
1723	0	0	0	22.0
1720	11.5	11.5	6.5	26.0

4549 3616

1697	14.5	12	7.5	26.3
1723	0	0	0	27.1
1709	5.5	6	1.5	25.8
1708	3	3.5	2	21.0

8968 3616

1875	17.5	16.5	7	22.3
1876	14	10.5	10	24.9
1877	11.5	10	11	25.5
1878	15	15	10	22.0

8972 RT

1859	9.5	10	0	22.8
1860	13	11.5	6	19.0
1861	10	12	5	17.1
1862	9	8	3.5	23.3

8964 RT

[illegible]9	7	7	5.5	19.0
1892	12.5	12.5	7	23.1
1893	10.5	9.5	7	22.4
1894	9	5.5	5	21.4

8969 891-6 + RT

1871	17	17	5	23.7
1872	0	6	4	22.2
1873	8	2.5	5.5	22.2
1874	8.5	7.5	7	26.0

8967 891-6 + RT

1879	0	0	0	24.5
1880	3.5	3	1	20.2
1881	5	6.5	2	23.9
1882	6.5	3.5	3.5	21.7

8971 3616 + RT

1863	9.5	8	3.5	19.7
1864	11	7	3	23.0
1865	7	5.5	3.5	24.4
1866	7	5.5	4	25.5

8960 3616 + RT

1883	4.5	7.5	1.5	20.7
1884	8.5	10	5	20.6
1885	10	8.5	6	19.6
1886	4	4	2	16.5

8968 10^? 3616 427

				wt			
1875	13.5	12	7	20.6	15.5	14.5	9.5
1876	12	10.5	8	21.3	13.5	12	[illegible]
1877	10	11	7	23.7	10.5	8.5	7.5
1878	10	8	6	24.3	11	9	[illegible]

8965 899-6 all mice drowned 2/28

				wt
1887	9	10.5	6	23.4
1888	10	7	5.5	24.0
1889	4	3.5	[illegible]	23.4
1890	6	6	4.5	22.4

8967 899-6 +RT

				wt			
1879	4	5	3	24.6	0	0	0
1880	3	3	2	19.5	0	0	0
1881	7	6	4	22.7	7	6	4
1882	7	6	4	20.0	6.5	4.5	3

[illegible]9 899-6 +RT

				wt			
1871	8	8	5	20.7	7	6	4.5
1872	8	4.5	4.5	20.6	8	:	4
1873	8.5	9	5.5	20.1	[illegible]	7.5	6
1874	10	8.5	5	23.6	8	7	4

8966 3616 +RT

				wt			
1883	5	4	3.5	20.6	4	3.5	3.5
1884	10	11	7.5	21.1	11	[illegible].5	6
1885	11	10	7.5	23.2	10.5	9	[illegible].5
1886	5	6	3.5	19.4	4	3.5	2.5

8964 RT

				wt.			
1891	7	7.5	6	20.1			
1892	9.5	9	7	24.0	7	7	6
1893	9	10	7.5	22.5	7	7.5	6.5
1894	9	9	[illegible]	21.8	11	9.5	7

File | Edit Data | Linear Regression | Set fx= | X

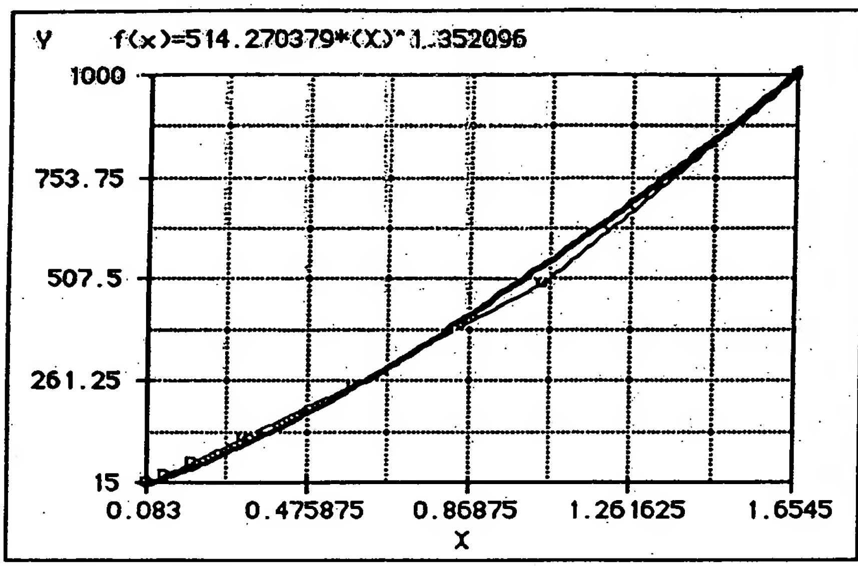
DATA:

X	Y
0.083	15.625
0.128	31.25
0.1925	62.5
0.318	125
0.587	250
1.0455	500
1.6545	1000

• EXPONENTIAL FIT •

Y= 514.270379*(X)^1.352096

Coef. of Regression= 0.995716 For 7 Data Pairs



INITIALS GSS SUBJECT • Untilted

DATE 1/26/95 CPU Time: 12 Secs.

TNF ELISA 1/26/95 U-87 Cells

reading 1	reading 2	average	[TNF]/ml	Time	Sample	[TNF]	Total [TNF]	Rel. [TNF]
0.053	0.063	0.058	10.9483	4 hours	control-super	41.0337	131.644	1497.08
0.053	0.052	0.0525	9.56856	4 hours	control-super			
0.096	0.114	0.105	24.4253	4 hours	control-pellet	90.6103		
0.089	0.098	0.0935	20.8799	4 hours	control-pellet			
0.717	0.721	0.719	329.222	4 hours	9 Gy-super	1275.75	1628.722	
0.646	0.725	0.6855	308.655	4 hours	9 Gy-super			
0.281	0.271	0.276	90.2195	4 hours	9 Gy-pellet	352.969		
0.281	0.253	0.267	86.265	4 hours	9 Gy-pellet			
0.111	0.13	0.1205	29.4229	6 hours	control-super	103.653	305.0815	1957.19
0.09	0.107	0.0985	22.4036	6 hours	control-super			
0.179	0.195	0.187	53.2992	6 hours	control-pellet	201.429		
0.177	0.166	0.1715	47.4151	6 hours	control-pellet			
0.875	0.872	0.8735	428.33	6 hours	9 Gy-super	1776.22	2262.27	
0.889	0.952	0.9205	459.781	6 hours	9 Gy-super			
0.344	0.344	0.344	121.512	6 hours	9 Gy-pellet	486.047		
0.36	0.328	0.344	121.512	6 hours	9 Gy-pellet			
0.305	0.332	0.3185	109.495	8 hours	control-super	397.223	660.3809	1933.52
0.27	0.277	0.2735	89.1164	8 hours	control-super			
0.22	0.21	0.215	64.3647	8 hours	control-pellet	263.158		
0.222	0.222	0.222	67.2141	8 hours	control-pellet			
1.084	1.073	1.0785	569.592	8 hours	9 Gy-super	2237.86	2593.9	
1.068	1.032	1.05	549.337	8 hours	9 Gy-super			
0.28	0.259	0.2695	87.3588	8 hours	9 Gy-pellet	356.041		
0.308	0.246	0.277	90.6617	8 hours	9 Gy-pellet			

TNF ELISA

1/31/95

Standards

1	2	3	4	5
0	"	1693 (48°)	"	1701 (8)
15	"	1696	"	"
31	"	1699	"	1715
63	"	1700	"	"
125	"	1703 (24°)	"	1718
250	"	1711	"	"
500	"	1712	"	1721
1000	"	1714	"	"

PLATE #:

ASSAY: DATE:

OPERATOR: O.D. LIMIT: [illegible] TIME:

WAVELENGTH: [illegible] READ MODE: OPTICAL DENSITY AUTO MIX: [illegible]

NOTES: CAL:

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.004	[illegible]	0.220	[illegible]	[illegible]	[illegible]	[illegible]	0.000	[illegible]	-0.000	0.000	0.000
B	0.132	[illegible]	0.117	0.114	0.462	-0.000	0.000	0.000	-0.000	-0.000	0.000	0.000
C	0.196	0.188	1.271	0.470	0.148	-0.000	0.000	-0.000	-0.000	0.000	0.000	0.000
D	0.248	0.330	2.667	1.356	0.106	-0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.382	0.337	1.774	0.936	1.137	-0.000	-0.000	0.000	0.000	0.000	0.000	0.000
F	0.619	0.627	0.788	0.319	0.331	[illegible]	[illegible]	0.000	0.000	0.000	-0.000	0.000
G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H	[illegible]	[illegible]	[illegible]	0.272	1.470	[illegible]	[illegible]	[illegible]	0.000	[illegible]	0.000	[illegible]

$$y = 648.842 \times x^{1.84}$$

TNF ELISA 1/31/95 In vivo

reading 1	reading 2	average	[TNF]/ml	Time	Mouse #
0.339	0.207	0.273	59.52191	48 hours	1693
0.117	0.114	0.1155	12.22611	48 hours	1696
1.271	0.47	0.8705	502.7038	48 hours	1699
2.667	1.356	2.0115	2347.548	48 hours	1700
1.744	0.936	1.34	1111.758	24 hours	1703
0.758	0.319	0.5385	207.7396	24 hours	1711
0.328	0.218	0.273	59.52191	24 hours	1712
0.974	0.379	0.6765	316.1043	24 hours	1714
0.85	0.462	0.656	298.7038	8 hours	1701
0.148	0.106	0.127	14.55916	8 hours	1715
1.197	0.631	0.914	549.8935	8 hours	1718
0.572	0.47	0.521	195.4875	8 hours	1721

TNF Interval (Infection with R899-6 to RT with 20 Gy)						
Mouse #	Tumor Size	Mean Size	Interval	[TNF]/ml	Mean [TNF]	[Prot]
1693	342	115.20	48 hours	59.52	730.50	
1696	62.5625		48 hours	12.23		
1699	45		48 hours	502.70		
1700	11.25		48 hours	2347.55		
1703	70	105.59	24 hours	1111.76	423.78	
1711	34.375		24 hours	207.74		
1712	255		24 hours	59.52		
1714	63		24 hours	316.10		
1701	90	56.56	8 hours	298.70	264.66	
1715	27.5		8 hours	14.56		
1718	41.25		8 hours	549.89		
1721	67.5		8 hours	195.49		

Infect $\xrightarrow{\text{I}}$ RT 20Gy $\xrightarrow{24°}$ SEC $\xrightarrow{20 Gy}$

I $\xrightarrow{24°}$ RT 20Gy $\xrightarrow{24°}$ RT 25Gy $\xrightarrow{\text{follow}}$

What we have:

① 4 mice injected c̄ 10^6 U87 cells, now c̄ small tumors
② 27 mice implanted c̄ 1 mm^3 U87 implants
③ 4 mice s̄ tumors injected c̄ R3616
④ 4 " " " " c̄ R899-6

I. Compare 3616 & 899-6 in mice c̄ 1° U87 implants c̄
- Design: alternately inject tumors c̄ 4×10^7 PFU of R3616 or R899-6 when tumors reach 6 mm in greatest dimension.
  Measure tumors 2x/wk
  Sac mice (i.e. 1 from each group on days 7, 14, 21, 28) for pathology?

II. Use 2° passaged U87 implants (Need 48 mice - order 60)
- Control + Saline
- RT (45 Gy single dose) + Saline
- R3616 (4×10^7 PFU single dose)
- R899-6 (4×10^7 PFU single dose)
- RT (45 Gy) + R3616
- RT (45 Gy) + R899-6

2×10^9/ml
.025
4×10^7

- Design: Take mice in groups of 4 when they reach 6mm in greatest dimension and randomly assign to a group. Use 8 mice per group.
- Deliver RT 24° (?) p̄ viral inoculation
- Measure tumors 2x/wk

III. In Vivo TNF assay $\bar{c}$ 2° passaged U87 tumors

- R3616
- R899-6
- R3616 + RT
- R899-6 + RT
- Design = inject tumors when reach 6 mm
  Sac mice & assay tumors for TNF at specific interva
  following inoculation: 12°, 24°, ~~36~~, 48°, 60°
  (Correct for [protein])

IV. Use 4 mice $\bar{c}$ small tumors inject $\bar{c}$ cells ①
- Sac & assay tumor for TNF to serve as
  control for III.

IN VIVO ~~U87~~ - R899-6, R3616, +/- RT
2/1/95

PURPOSE - To compare effects of R899-6 & R3616 virus, (on U87 xenografts in hind limb of nude mice), with and without RT vs RT alone

METHOD

1.) Implant approx 1 mm^3 pieces of ~~U87~~ minced U87 tumor (taken from previous mouse c̄ tumor) into rig. hind limb of nude mice. Specifically: ① Sac mouse bearing tumor & excise tumor. ② Place in 100 mm tissue culture dish containing culture media & mince into small pieces using scalpel.
③ Anesthetize mice with 0.3 - 0.4 ml of Ketamine / Xylazine preparation
④ Paint Ⓡ hind limb with tincture of benzoin
⑤ Make small nick in Ⓡ hind limb with #11 blade scalp.
⑥ Open subcut space with forceps
⑦ Insert tumor implant into pouch c̄ forceps
⑧ Apply small steri-strip to close wound

2.) Use mice when tumors reach 100 mm^3 (≥ 2 weeks) & ~~randomize to~~ assign to treatment group

3.) For all groups: measure tumors twice weekly (M & Th) weigh mice weekly (M) & monitor for adverse eff. sac mice when tumors reach , or when sy...

4.) RT given on T & Th = 20 Gy (induction dose) & 25...

5.) Infection delivered via 1 injection (3 sites) on Monday (24° prior to first RT dose)

TNF U87

2/1/95

PURPOSE - To optimize intervals in order to test for RT inducibility

METHODS -

① U87 cells were grown to subconfluence in 60mm plates
② Cell were infected with 10^6 PFU of R899-6 in 1.5 ml 199V media for 2°, then 2.5 ml of U87 media + IgG was added (total vol 4 ml)
③ Cells were incubated and irradiated with 8 Gy at the following intervals. The "supernatant" and "pellet" samples were aliquotted as in previous experiments.

Infect @ 6 AM ⇒

RT time	Assay time
None	6, 8, 10, 12, 14°
6°	8, 10, 12, 14°
8°	10, 12, 14°
10°	12, 14°
12°	14°

④ Prepare samples by freeze thaw x 3, then centrifuge
⑤ Measure human TNFα by ELISA

1	2	3	4	5	6	7	8	9	10
0	"	T_6 s	"	R_8T_{10} s	"	R_8T_{12} s	"	$R_{10}T_{14}$ s	"
15	"	T_6 p	"	R_8T_{10} p	"	R_8T_{12} p	"	$R_{10}T_{14}$ p	"
31	"	T_8 s	"	R_6T_{10} s	"	R_6T_{12} s	"	R_8T_{14} s	"
63	"	T_8 p	"	R_6T_{10} p	"	R_6T_{12} p	"	R_8T_{14} p	"
125	"	R_6T_8 s	"	T_{12} s	"	T_{14} s	"	R_6T_{14} s	"
250 *	"	R_6T_8 p	"	T_{12} p	"	T_{14} p	"	R_6T_{14} p	"
500	"	T_{10} s	"	$R_{10}T_{12}$ s	"	$R_{12}T_{14}$ s	" *		
1000	"	T_{10} p	"	$R_{10}T_{12}$ p	"	$R_{12}T_{14}$ p	"		

MOLECULAR DEVICES

ASSAY: TNF

DATE: 2 2 95

OPERATOR: Sibley/Hyland

O.D. LIMIT: [illegible]

TIME: [illegible]

WAVELENGTH: [illegible]

READ MODE: OPTICAL DENSITY

AUTO MIX: [illegible]

NOTES:

CAL

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.041	0.048	0.107	0.105	0.257	0.260	0.700	0.795	0.830	0.855	-0.000	[illegible]
B	0.113	0.110	0.226	0.240	0.124	[illegible]	[illegible]	[illegible]	1.308	[illegible]	[illegible]	[illegible]
C	0.157	[illegible]	[illegible]	0.253	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
D	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

$$y = 250.46 * x^{1.1968}$$

File | Edit Data | Linear Regression | Set fx= X

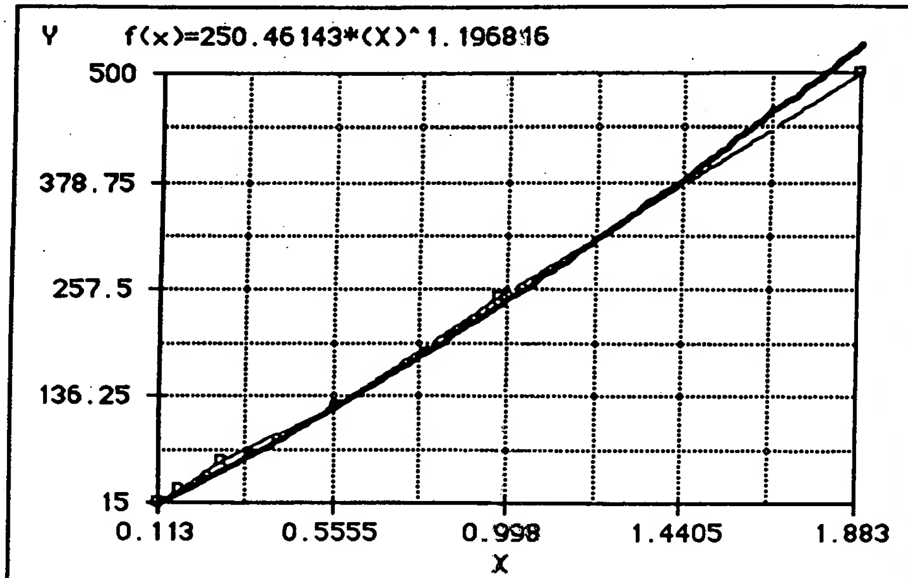
DATA:

X	Y
0.113	15.625
0.1645	31.25
0.2785	62.5
0.5645	125
0.981	250
1.883	500

• EXPONENTIAL FIT •

Y= 250.46143*(X)^1.196816

Coef. of Regression= 0.993049 For 6 Data Pairs



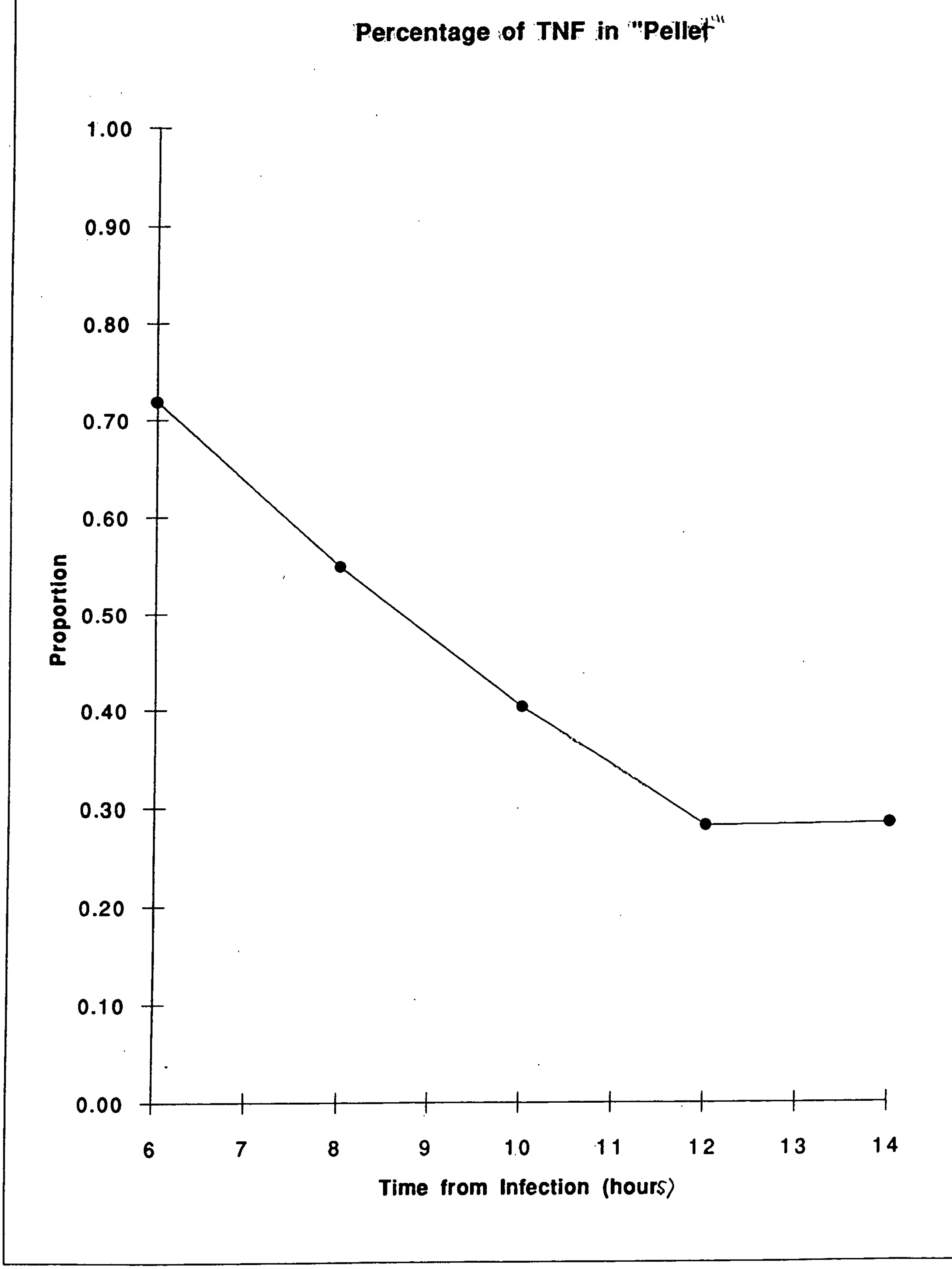
INITIALS GSS SUBJECT • Untilted

DATE 2/2/95

CPU Time: 11 Secs.

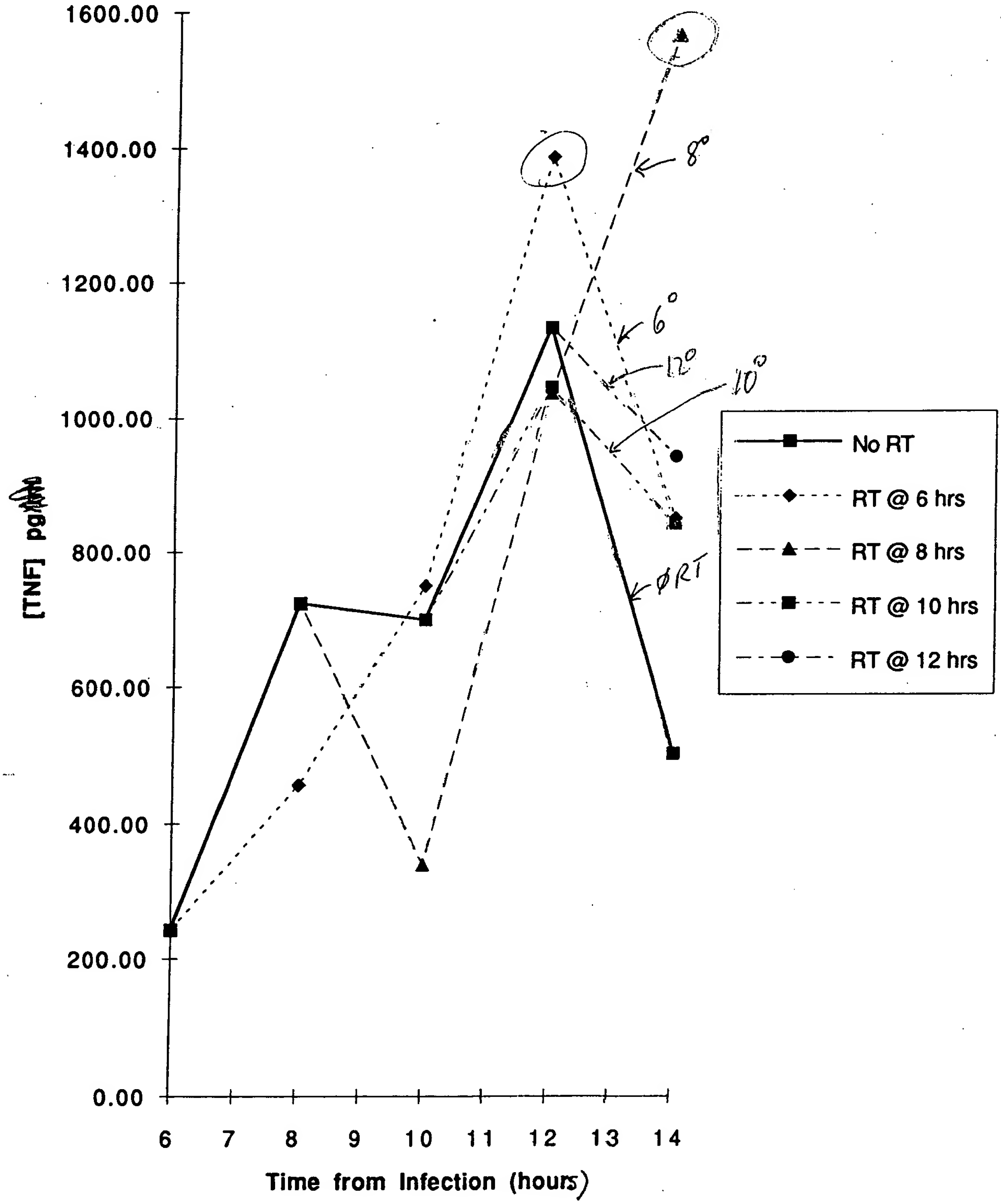
TNF ELISA 2/2/95 U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Total [TNF]
0.107	0.105	0.106	17.07	None	6 hours	supernatant	68.28	243.08
0.225	0.24	0.2325	43.70	None	6 hours	pellet	174.80	
0.389	0.395	0.392	81.66	None	8 hours	supernatant	326.62	723.19
0.46	0.462	0.461	99.14	None	8 hours	pellet	396.57	
0.294	0.299	0.2965	58.46	6 hours	8 hours	supernatant	233.84	456.40
0.281	0.288	0.2845	55.64	6 hours	8 hours	pellet	222.56	
0.484	0.478	0.481	104.31	None	10 hours	supernatant	417.24	699.51
0.349	0.345	0.347	70.57	None	10 hours	pellet	282.27	
0.267	0.262	0.2645	50.99	8 hours	10 hours	supernatant	203.97	339.52
0.188	0.188	0.188	33.89	8 hours	10 hours	pellet	135.55	
0.514	0.534	0.524	115.57	6 hours	10 hours	supernatant	462.27	749.90
0.339	0.366	0.3525	71.91	6 hours	10 hours	pellet	287.63	
0.817	0.865	0.841	203.58	None	12 hours	supernatant	814.32	1134.47
0.379	0.392	0.3855	80.04	None	12 hours	pellet	320.15	
0.755	0.767	0.761	180.63	10 hours	12 hours	supernatant	722.50	1046.13
0.388	0.39	0.389	80.91	10 hours	12 hours	pellet	323.63	
0.709	0.795	0.752	178.07	8 hours	12 hours	supernatant	712.29	1038.41
0.374	0.409	0.3915	81.53	8 hours	12 hours	pellet	326.12	
0.974	1.067	1.0205	256.62	6 hours	12 hours	supernatant	1026.47	1387.28
0.422	0.43	0.426	90.20	6 hours	12 hours	pellet	360.81	
0.431	0.419	0.425	89.95	None	14 hours	supernatant	359.79	503.15
0.196	0.198	0.197	35.84	None	14 hours	pellet	143.36	
0.72	0.737	0.7285	171.43	12 hours	14 hours	supernatant	685.73	943.84
0.316	0.328	0.322	64.53	12 hours	14 hours	pellet	258.11	
0.639	0.655	0.647	148.74	10 hours	14 hours	supernatant	594.96	843.50
0.308	0.316	0.312	62.14	10 hours	14 hours	pellet	248.54	
1.174	1.202	1.188	307.81	8 hours	14 hours	supernatant	1231.23	1566.84
0.399	0.403	0.401	83.90	8 hours	14 hours	pellet	335.62	
0.68	0.661	0.6705	155.23	6 hours	14 hours	supernatant	620.92	852.40
0.284	0.304	0.294	57.87	6 hours	14 hours	pellet	231.48	



Percentage of TNF in "Pellet"
Proportion
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
6
7
8
9
10
11
12
13
14
Time from Infection (hours)

TNF production by interval to RT



THE UNIVERSITY OF CHICAGO
DEPARTMENT OF RADIATION & CELLULAR ONCOLOGY
DIVISION OF THE BIOLOGICAL SCIENCES AND
THE PRITZKER SCHOOL OF MEDICINE

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Main Office: (312) 702-6819
Appointment Desk: (312) 702-6860
Facsimile: (312) 702-0610

Date: 2/6/95

To: James Linsley

From: ~~Helena J. Mauceri~~ Gregory S. Sibley, MD

Please transfer the cages listed below from CLSC 1053 to Carlson room ~~J-019~~ J-013.
Please have the transfer completed by 5:00 p.m. 2/7/95.
If this is not possible please call me at 2-0492.

Thank you.

Greg Sibley BP #3439
EXT 2-0294

Cage numbers:

~~1859~~

~~AA108958 - AA108964~~ (7 cages)

AA10966 - AA 108972

Mouse # 1859 - 1886

TNF U87 in vitro

2/6/95

1	2	3	4	5	6	7	8	9

PURPOSE – To determine whether TNF production is ~~Prod~~ induced with 9 Gy RT following R899-6 infection (10^6 PFU = ~ MOI = 0.5) in U87 cells.

METHODS – From previous experiments the optimal interval from infection to RT was 6 hrs. ~~And~~ optimal interval following RT was 6-8 hrs (total interval 12-14 hrs from infection.) ..

1. See methods from 2/1/95. Briefly, 10^6 PFU of R899-6 was placed in 60 mm plates of subconfluent (~ 2×10^6 cells) U87 cells & incubate x2° in 199V media @ 37°.
2. 10% FCS Media + IgG added @ 2°
3. 9 Gy RT given @ 6° to RT group.
4. Plates sampled at 12 & 14° for "supernatant" & "pellet"

PLATE #

BODY: TNF - U87 DATE: 2 7 95

OPERATOR: Sibley / Hyland O.D. LIMIT: TIME:

WAVELENGTH: READ MODE: AUTO MIX:

$y = 216.779 \times x^{1.154631}$

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
	.0495											
A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
B	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
C	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
D	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
E	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
H	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

O.D. LIMIT

WAVELENGTH: READ MODE: AUTO MIX

OPTICAL DENSITY

1	2	3	4	5	6	7	8	9	10	11	12

1	2	3	4	5	6	7	8	9
1-2	3-4	5-6	7-8	9-10	11-12	13-14		
0	12 S	12 P	12 RT S	14 S	14 P	"		
15	12 S	12 P	12 RT S	"	"	"		
31	12 S	12 P	12 RT P	"	"	14 RT P		
63	12 S	12 P	12 RT P	"	"	"		
125	12 S	12 RT S	12 RT P	"	14 RT S	"		
250	12 S	12 RT S	12 RT P	"	"	"		
500	12 P	12 RT S	12 RT P	14 P	"	"		
1000	12 P	12 RT S	12 RT P	"	"	"		

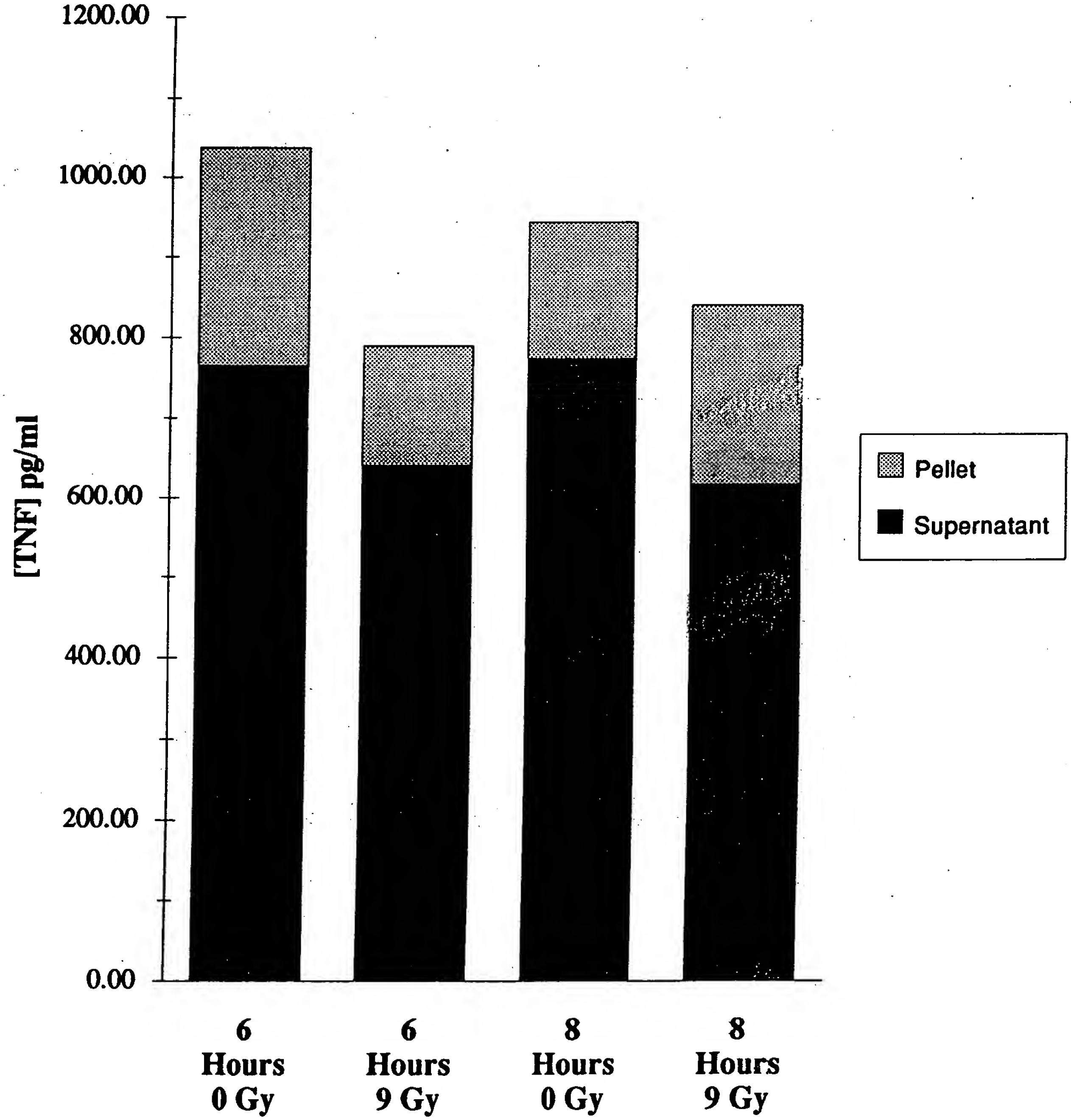
3 14
×8
112
×2
224

TNF ELISA 2/2/95 U-87 Cells

reading 1	reading 2	average	[TNF]/ml	RT Time	Sample Time	Type	[TNF]	Ave [TNF]	Total TNF
0.908	0.896	0.902	192.44	none	12 hours	supernatant	769.76	764.69	1038.11
0.846	0.748	0.797	166.82	none	12 hours	supernatant	667.26		
0.892	0.847	0.8695	184.46	none	12 hours	supernatant	737.83		
0.857	0.842	0.8495	179.57	none	12 hours	supernatant	718.27		
0.936	0.945	0.9405	201.96	none	12 hours	supernatant	807.82		
1.014	1.026	1.02	221.79	none	12 hours	supernatant	887.17		
0.368	0.372	0.37	68.78	none	12 hours	pellet	275.11	273.43	
0.383	0.386	0.3845	71.90	none	12 hours	pellet	287.60		
0.363	0.36	0.3615	66.96	none	12 hours	pellet	267.83		
0.392	0.377	0.3845	71.90	none	12 hours	pellet	287.60		
0.366	0.33	0.348	64.08	none	12 hours	pellet	256.31		
0.368	0.351	0.3595	66.53	none	12 hours	pellet	266.12		
0.824	0.884	0.854	180.67	6 hours	12 hours	supernatant	722.66	640.45	789.94
0.791	0.835	0.813	170.69	6 hours	12 hours	supernatant	682.76		
0.833	0.821	0.827	174.09	6 hours	12 hours	supernatant	696.35		
0.734	0.721	0.7275	150.14	6 hours	12 hours	supernatant	600.54		
0.693	0.8	0.7465	154.67	6 hours	12 hours	supernatant	618.69		
0.602	0.686	0.644	130.42	6 hours	12 hours	supernatant	521.69		
0.234	0.251	0.2425	42.23	6 hours	12 hours	pellet	168.91	149.49	
0.188	0.219	0.2035	34.49	6 hours	12 hours	pellet	137.95		
0.21	0.241	0.2255	38.83	6 hours	12 hours	pellet	155.31		
0.234	0.222	0.228	39.33	6 hours	12 hours	pellet	157.30		
0.209	0.21	0.2095	35.66	6 hours	12 hours	pellet	142.66		
0.203	0.196	0.1995	33.71	6 hours	12 hours	pellet	134.82		
0.876	0.93	0.903	192.69	none	14 hours	supernatant	770.75	774.25	943.63
0.813	0.792	0.8025	168.15	none	14 hours	supernatant	672.58		
0.935	0.947	0.941	202.08	none	14 hours	supernatant	808.32		
0.924	0.9	0.912	194.91	none	14 hours	supernatant	779.63		
0.969	0.953	0.961	207.05	none	14 hours	supernatant	828.19		
0.924	0.913	0.9185	196.51	none	14 hours	supernatant	786.04		
0.248	0.249	0.2485	43.44	none	14 hours	pellet	173.74	169.38	
0.27	0.271	0.2705	47.90	none	14 hours	pellet	191.62		
0.251	0.254	0.2525	44.24	none	14 hours	pellet	176.97		
0.233	0.24	0.2365	41.02	none	14 hours	pellet	164.09		
0.244	0.224	0.234	40.52	none	14 hours	pellet	162.09		
0.211	0.221	0.216	36.95	none	14 hours	pellet	147.78		
0.65	0.653	0.6515	132.18	6 hours	14 hours	supernatant	528.71	616.03	840.68
0.722	0.696	0.709	145.74	6 hours	14 hours	supernatant	582.95		
0.62	0.555	0.5875	117.30	6 hours	14 hours	supernatant	469.21		
0.775	0.784	0.7795	162.59	6 hours	14 hours	supernatant	650.38		

0.886	0.814	0.85	179.69	6 hours	14 hours	supernatant	718.76	
0.895	0.861	0.878	186.54	6 hours	14 hours	supernatant	746.16	
0.314	0.294	0.304	54.82	6 hours	14 hours	pellet	219.27	224.66
0.307	0.303	0.305	55.03	6 hours	14 hours	pellet	220.11	
0.296	0.31	0.303	54.61	6 hours	14 hours	pellet	218.44	
0.295	0.307	0.301	54.19	6 hours	14 hours	pellet	216.78	
0.315	0.312	0.3135	56.80	6 hours	14 hours	pellet	227.20	
0.343	0.329	0.336	61.53	6 hours	14 hours	pellet	246.14	

TNF Production By RT in U87 Cells Following R899-6 Infection



Titers 2/14/95

New samples (2/13/95) = Virus used for all in vivo exper.

Virus	Dilution	Count		Titer
3616	10^7	TM	TM	
	10^8	23	28	2.6×10^9
899-6	10^7	TM	TM	
	10^8	29	47	3.8×10^9

Old samples (used for in vitro exper.)

Virus	Dilution	Count		Titer
3616	10^7	TM	TM	
	10^8	45	36	4.1×10^9
899-6	10^7	TM	TM	
	10^8	35	26	3.1×10^9

2/16/97

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1704	AA104550	Control	21	21	11	0	21.7				0	
1717	AA104550	Control	13	12.5	10.5	0					0	
1719	AA104550	Control	13	11.5	6	0					0	
1722	AA104550	Control	14	13.5	10	0					0	
1867	AA108970	Control	-10	8	6							
1868	AA108970	Control	-10	9.5	7.5							
1869	AA108970	Control	-10	9	7							
1870	AA108970	Control	-10.5	8.5	8							
		Control		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1720	AA105283	10^7 899-6	12	11	7.5	0					0	
1710	AA105283	10^7 899-6	16	13.5	7	0					0	
1707	AA105283	10^7 899-6	16	15	11	0	17.2				0	
1713	AA105283	10^7 899-6	6.5	5.5	3	0					0	
		10^7 899-6		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1697	AA104549	10^7 3616	-10	9	7	0					0	
1708	AA104549	10^7 3616	8	8.5	5	0					0	
1709	AA104549	10^7 3616	9	10	7	0					0	
1723	AA104549	10^7 3616	7.5	9	6	0					0	
		10^7 3616		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1859	AA108972	RT Alone	9.5	9.5	5.5	0					0	
1860	AA108972	RT Alone	12	10.5	8	0					0	
1861	AA108972	RT Alone	10.5	9	7	0					0	
1862	AA108972	RT Alone	12	9	7	0					0	
		RT Alone		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	

1863	AA108971	10^7 3616+RT	9	8	6.5	0					0	
1864	AA108971	10^7 3616+RT	+12.5	7.5	6	0					0	
1865	AA108971	10^7 3616+RT	10	9	6.5	0					0	
1866	AA108971	10^7 3616+RT	-10.5	7.5	5.5	0					0	
1883	AA108966	10^7 3616+RT	7.5	7	5	0					0	
1884	AA108966	10^7 3616+RT	11.5	10	8	0					0	
1885	AA108966	10^7 3616+RT	12	9	7	0					0	
1886	AA108966	10^7 3616+RT	7.5	7.5	5	0					0	
		10^7 3616+RT		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		0			Day:		0	
1871	AA108969	10^7 899-6+RT	9	8	5.5	0					0	
1872	AA108969	10^7 899-6+RT	9.5	7.5	6.5	0					0	
1873	AA108969	10^7 899-6+RT	+9.5	8	7	0					0	
1874	AA108969	10^7 899-6+RT	11	10.5	8	0					0	
1879	AA108967	10^7 899-6+RT	+8	9	6	0					0	
1880	AA108967	10^7 899-6+RT	8	7	6	0					0	
1881	AA108967	10^7 899-6+RT	8	7	5	0					0	
1882	AA108967	10^7 899-6+RT	9.5	8	5	0					0	
		10^7 899-6+RT		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		0			Day:		0	
1887	AA108965	10^7 894-6	8.5	6	5		22.5					
1888	AA108965	"	8.5	5.5	4.5		23.4					
1889	AA108965	"	5.5	8	5		22.8					
1890	AA108965	"	7.5	6.5	5		23.3					
				Mean:		#DIV/0!			Mean:		#DIV/0!	
				Std Error:		#DIV/0!			Std Error:		#DIV/0!	
				Day:		0			Day:		0	
1875	AA108968											
1876	AA108968											
1877	AA108968											
1878	AA108968											
				Mean:		#DIV/0!			Mean:		#DIV/0!	

				Std Error:		#DIV/0!			Std Error:		#DIV/0!	
				Day:		0			Day:		0	
1891	AA108964											
1892	AA108964											
1893	AA108964											
1894	AA108964											
1895	AA108963											
1896	AA108963											
1897	AA108963											
1898	AA108963											
1899	AA108962											
1900	AA108962											
1901	AA108962											
1902	AA108962											
1903	AA108961											
1904	AA108961											
1905	AA108961											
1906	AA108961											
1907	AA108960											
1908	AA108960											
1909	AA108960											
1910	AA108960											
1911	AA108959											
1912	AA108959											
1913	AA108959											
1914	AA108959											
1915	AA108958											
1916	AA108958											
1917	AA108958											
1918	AA108958											
1702	AA104544											
1724	AA104544											

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 18781

ARC USE ONLY

P.O. # ____

ORDER DATE ____

REF. # ____

CONTACT ____

EST AMT ____

SCHED DEL ____

SPECIAL ROUTING

NON COM VENDOR ____

FLAGGED BY PROTOCOL ____

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ____ SHIPMENTS ON A ____ BASIS

REQUEST BY: Greg Sibley, MD DATE: 2/16/95

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: ____

FAS ACCOUNT: 2-7371-5100

P.I.: Hallahan

VENDOR: FCRI

PROTOCOL: 58671

REQUESTED DELIVERY DATE: 2/21/95

PHONE: 2-0294

SPECIES: Mouse

QUANTITY: 60

STRAIN: Athymic Nude

SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE: ____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐
(RODENTS ONLY)

SPECIAL REQUIREMENTS: ____

HOUSE AT: ____ CARLSON ____ WYLER X CLSC ____ FMI ____ OTHER

The University of Chicago Departmental Purchase Order

Greg

Purchase Order Number
Z 873342
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
USA Orders - Cust #112486
Life Technologies, Inc
P.O. Box 68
Grand Island | NY | 14072
City | State | Zip Code
Payment Terms ______ Delivery charge? ☐ Yes ☒ No
Telephone No. 1-800-828-6686 FAX No. 1-800-331-2286

Ship To
THE UNIVERSITY OF CHICAGO
Dept. Code: ______
Radiation Oncology MC-0085
Department
Greg Sibley, MD 6-03
Attn: Room
5830 S. Ellis Ave
Chicago | IL | 60637
City | State | Zip Code

372-369

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of **$500.00** for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? ☐ No ☐ Yes
Melissa Ref #372369 | 2/17/95
Order placed with (name) | Date
Greg Sibley, MD
Order placed by (name) | Phone #

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
24		11095-023 Minimal Essential Media 500 ml	8.64	207.36
12 ~~25~~		15140-015 Penicillin - Streptomycin 100 ml	10.00	~~250.00~~ 120.00
~~18~~		~~22400-055 RPMI 1640 500 ml~~		
12		22400-055 RPMI 1640 Media 500 ml	12.91	154.92
			ORDER TOTAL	482.28

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00

BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

2/25/95

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
~~1704~~	~~AA104550~~	~~Control~~										
1717	AA104550	Control.	18	15.5	14	0					0	
1719	AA104550	Control	12	15	8	0					0	
1722	AA104550	Control	19.5	18	13	0					0	
1867	AA108970	Control	13	16	9.5	0					0	
1868	AA108970	Control	18	15	13							
1869	AA108970	Control	15.5	16	9							
1870	AA108970	Control	15.5	13	11							
		Control		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1720	AA105283	10^7 899-6	11.5	11.5	8	0					0	
1710	AA105283	10^7 899-6	19.5	16	8	0					0	
1707	AA105283	10^7 899-6	19	16.5	13	0					0	
1713	AA105283	10^7 899-6	∅			0					0	
		10^7 899-6		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1697	AA104549	10^7 3616	11	6.5	5.5	0					0	
1708	AA104549	10^7 3616	5.5	7	3.5	0					0	
1709	AA104549	10^7 3616	6	7.5	4	0					0	
1723	AA104549	10^7 3616	8	9	5	0					0	
		10^7 3616		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	
1859	AA108972	RT Alone	10	11.5	6.5	0					0	
1860	AA108972	RT Alone	9	8	6.5	0					0	
1861	AA108972	RT Alone	-11.5	12.5	8	0					0	
1862	AA108972	RT Alone	-11	9	4	0					0	
		RT Alone		Mean:		0			Mean:		0	
				Std Error:		0			Std Error:		0	
				Day:		17			Day:		0	

March 8, 1995

Jim Linsley
P-110
Animal Resources

Dear Jim,

Please transfer the cages listed below from CLSC 1053 to Carlson room **J-O13**.
Please have the transfer completed by 5 pm on **3/10/95**.

If this is not possible, please contact me.

Thank you,

Greg Sibley, M.D.
Dept. of Radiation and Cellular Oncology
Beeper #3439
Extension 2-0294

Cage Numbers:
AA111326 through AA111333

Total Number of Cages: **8**

3/9/95

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6	17.5	12	9.5							
1710	AA105283	10^7 899-6	6.5	6.5	1.5							
1713	AA105283	10^7 899-6	0	0	0							
		10^7 899-6										
1697	AA104549	10^7 3616	15.5	13.5	9							
1708	AA104549	10^7 3616	2	2.5	1.5							
1709	AA104549	10^7 3616	5	5	1							
1723	AA104549	10^7 3616	0	0	0							
1875	AA108968	10^7 3616	19	18.5	10							
1876	AA108968	10^7 3616	18	17	11.5							
1877	AA108968	10^7 3616	18.5	14	13							
1878	AA108968	10^7 3616	16	12.5	11							
		10^7 3616										
1859	AA108972	RT Alone	9	1.5	4							
1860	AA108972	RT Alone 13x11x6	~~8.5~~	~~7.5~~	~~5~~							
1861	AA108972	RT Alone	10	10.5	7							
1862	AA108972	RT Alone	8.5	7.5	3							
1891	AA108964	RT Alone	6.5	6.5	5							
1892	AA108964	RT Alone	12.5	9.5	6							
1893	AA108964	RT Alone	10	9.5	5.5							
1894	AA108964	RT Alone	8	8	5							
		RT Alone										
1863	AA108971	10^7 3616+RT	10	8	4							
1864	AA108971	10^7 3616+RT	10.5	7	4							
1865	AA108971	10^7 3616+RT	5.5	6.5	4.5							
1866	AA108971	10^7 3616+RT	7	5	4.5							
1883	AA108966	10^7 3616+RT	3.5	3	1.5							
1884	AA108966	10^7 3616+RT	8	9	6							
1885	AA108966	10^7 3616+RT	9	8.5	4							
1886	AA108966	10^7 3616+RT	3.5	4	2							
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT	7.5	7	4.5							
1872	AA108969	10^7 899-6+RT	7	5.5	4.5							
1873	AA108969	10^7 899-6+RT	8	7.5	4.5							
1874	AA108969	10^7 899-6+RT	8	7.5	4							
1879	AA108967	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	3.5	3	1							
1881	AA108967	10^7 899-6+RT	5.5	6	3							
1882	AA108967	10^7 899-6+RT	4	3	1							
		10^7 899-6+RT										
2032	AA111326	10^7 899-6										
2033	AA111326	10^7 899-6										
2034	AA111326	10^7 899-6										
2035	AA111326	10^7 899-6										
2036	AA111327	10^7 899-6										
2037	AA111327	10^7 899-6										
2038	AA111327	10^7 899-6										
2039	AA111327	10^7 899-6										
2040	AA111328	10^7 899-6										
2041	AA111328	10^7 899-6										
2042	AA111328	10^7 899-6										
2043	AA111328	10^7 899-6										
		10^7 899-6										
2044	AA111329	10^7 3616										
2045	AA111329	10^7 3616										
2046	AA111329	10^7 3616										
2047	AA111329	10^7 3616										
2048	AA111330	10^7 3616										
2049	AA111330	10^7 3616										
2050	AA111330	10^7 3616										
2051	AA111330	10^7 3616										
		10^7 3616										
2052	AA111331	10^7 899-6+RT										
2053	AA111331	10^7 899-6+RT										
2054	AA111331	10^7 899-6+RT										
2055	AA111331	10^7 899-6+RT										
2056	AA111332	10^7 899-6+[illegible]										
2057	AA111332	10^7 899-[illegible]										
2058	AA111332	10^7 899[illegible]										
2059	AA111332	10^7 899[illegible]										
		10^7 899-6+[illegible]										

2060	AA111333	10^7 3616+RT										
2061	AA111333	10^7 3616+RT										
2062	AA111333	10^7 3616+RT										
2063	AA111333	10^7 3616+RT										
2067	AA111335	10^7 3616+RT										
2068	AA111335	10^7 3616+RT										
2069	AA111335	10^7 3616+RT										
2070	AA111335	10^7 3616+RT										
		10^7 3616+RT										

3/13/95

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6										
1710	AA105283	10^7 899-6										
1713	AA105283	10^7 899-6										
		10^7 899-6										
1697	AA104549	10^7 3616										
1708	AA104549	10^7 3616										
1709	AA104549	10^7 3616										
1723	AA104549	10^7 3616										
1875	AA108968	10^7 3616										
1876	AA108968	10^7 3616										
1877	AA108968	10^7 3616										
1878	AA108968	10^7 3616										
		10^7 3616										
1859	AA108972	RT Alone										
1860	AA108972	RT Alone										
1861	AA108972	RT Alone										
1862	AA108972	RT Alone										
1891	AA108964	RT Alone										
1892	AA108964	RT Alone										
1893	AA108964	RT Alone										
1894	AA108964	RT Alone										
		RT Alone										
1863	AA108971	10^7 3616+RT										
1864	AA108971	10^7 3616+RT										
1865	AA108971	10^7 3616+RT										
1866	AA108971	10^7 3616+RT										
1883	AA108966	10^7 3616+RT										
1884	AA108966	10^7 3616+RT										
1885	AA108966	10^7 3616+RT										
1886	AA108966	10^7 3616+RT										
		10^7 3616+RT										

3/13/95

~~1871~~	~~AA108969~~	~~10^7 899-6+RT~~										
1872	AA108969	10^7 899-6+RT										
~~1873~~	~~AA108969~~	~~10^7 899-6+RT~~										
1874	AA108969	10^7 899-6+RT										
1879	AA108967	10^7 899-6+RT										
1880	AA108967	10^7 899-6+RT										
1881	AA108967	10^7 899-6+RT										
1882	AA108967	10^7 899-6+RT										
		10^7 899-6+RT										
2032	AA111326	10^7 899-6 +RT	9	7	6		22.2	3616	+RT			
2033	AA111326	10^7 899-6 [illegible]	11	7.5	6.5		22.6	"				
2034	AA111326	10^7 899-6 [illegible]	11	10	7		19.6	"				
2035	AA111326	10^7 899-6 [illegible]	12.5	7	6		19.0	"				
2036	AA111327	10^7 899-6 +RT	10	7	5		19.7	3616	+RT			
2037	AA111327	10^7 899-6 [illegible]	8	7	4.5		21.7	"				
2038	AA111327	10^7 899-6 [illegible]	11.5	9.5	7.5		13.0	"				
2039	AA111327	10^7 899-6 [illegible]	9.5	7	5		18.5	"				
2040	AA111328	10^7 899-6	11	9.5	7		21.3	3616				
2041	AA111328	10^7 899-6	6.5	6	5		21.3	~~899-6 +RT~~	~~"~~			
2042	AA111328	10^7 899-6	10	10	7.5		23.2	"				
2043	AA111328	10^7 899-6	8	6.5	5.5		23.7	"				
		10^7 899-6										
2044	AA111329	10^7 3616 +RT	8.5	7	4.5		19.3	899-6	+RT			
2045	AA111329	10^7 3616 +RT	7	6	4		22.4	"				
2046	AA111329	10^7 3616 "	6.5	6	5		20.7	"				
2047	AA111329	10^7 3616 "	9	8.5	5		21.5	"				
2048	AA111330	10^7 3616 +RT	8	5	4.5		19.2	899-6	+RT			
2049	AA111330	10^7 3616 "	11	7	5.5		22.6	"				
2050	AA111330	10^7 3616 "	8.5	6.5	5.5		21.1	"				
2051	AA111330	10^7 3616 "	8.5	8.5	5.5		19.0	"				
		10^7 3616										
2052	AA111331	10^7 899-6+RT	4	6	5		[illegible].4	899-6				
2053	AA111331	10^7 899-6+RT	7	6	4.5		[illegible]	899-6				
2054	AA111331	10^7 899-6+RT	7	6	4.5		20.1	"				
2055	AA111331	10^7 899-6+RT	11	6.5	5		13.3	"				
2056	AA111332	10^7 899-6+RT	8	5	4		21.7	899-6				
2057	AA111332	10^7 899-6+RT	7	6	4.5		21.9	"				
2058	AA111332	10^7 899-6+RT	7	9	5.5		22.9	"				
2059	AA111332	10^7 899-6+RT						"				
		10^7 899-6+RT										

extra injected

Bubba tumors

~~6.5~~ ~~6.5~~ ~~5.5~~ ~~22.4~~

1331 1 tumor

1332 2 [illegible]

2060	AA111333	10^7 3616+RT										
2061	AA111333	10^7 3616+RT										
2062	AA111333	10^7 3616+RT										
2063	AA111333	10^7 3616+RT										
2067	AA111335	10^7 3616+RT										
2068	AA111335	10^7 3616+RT										
2069	AA111335	10^7 3616+RT										
2070	AA111335	10^7 3616+RT										
		10^7 3616+RT										

Greg's Mouse Log

Mouse #	Cage #	Group	3/13/95			Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6	20.5	10	13	26.0						
1710	AA105283	10^7 899-6	0	0	0	23.0						
1713	AA105283	10^7 899-6	0	0	0	21.6						
		10^7 899-6										
1697	AA104549	10^7 3616	17.5	14.5	11	23.8						
1708	AA104549	10^7 3616	0	0	0	24.0						
1709	AA104549	10^7 3616	3.5	4	1	28.2						
1723	AA104549	10^7 3616	0	0	0	26.3						
1875	AA108968	10^7 3616	19	20	10	22.3						
1876	AA108968	10^7 3616	19	17	13.5	23.5						
1877	AA108968	10^7 3616	22.5	17.5	16.5	27.3						
1878	AA108968	10^7 3616	18.5	15.5	15.5	26.0						
		10^7 3616										
1859	AA108972	RT Alone	10 ~~3~~	10 ~~12~~	5 ~~8.0~~		25.1 ~~15.0~~					
1860	AA108972	RT Alone	13	1	8.5		8.7					
1861	AA108972	RT Alone	11	15	7		20.9					
1862	AA108972	RT Alone	9	7.5	3.5		24.7					
1891	AA108964	RT Alone	6.5	7.5	3.5		19.8					
1892	AA108964	RT Alone	10.5	9.5	6		21.5					
1893	AA108964	RT Alone	10	9	5.5		20.8					
1894	AA108964	RT Alone	7.5	8	4		21.5					
		RT Alone										
1863	AA108971	10^7 3616+RT	9.5	8	3.5	22.9						
1864	AA108971	10^7 3616+RT	6	5	4	25.3						
1865	AA108971	10^7 3616+RT	10	6.5	4	24.9						
1866	AA108971	10^7 3616+RT	5	4	3.5	25.1						
1883	AA108966	10^7 3616+RT	4	3	1	21.7						
1884	AA108966	10^7 3616+RT	8.5	7	4.5	23.5						
1885	AA108966	10^7 3616+RT	Dead	3/13/95								
1886	AA108966	10^7 3616+RT	3	4	2	22.5						
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT	8	7	5	23.3						
1872	AA108969	10^7 899-6+RT	12	5.5	4	22.3						
1873	AA108969	10^7 899-6+RT	8	7.5	4.5	23.3						
1874	AA108969	10^7 899-6+RT	8	8	4	26.1						
1879	AA108967	10^7 899-6+RT	0	0	0	25.0						
1880	AA108967	10^7 899-6+RT	0	0	0	21.3						
1881	AA108967	10^7 899-6+RT	5.5	5.5	2.5	24.0						
1882	AA108967	10^7 899-6+RT	4	3	1	22.5						
		10^7 899-6+RT										
2032	AA111326	10^7 899-6										
2033	AA111326	10^7 899-6										
2034	AA111326	10^7 899-6										
2035	AA111326	10^7 899-6										
2036	AA111327	10^7 899-6										
2037	AA111327	10^7 899-6										
2038	AA111327	10^7 899-6										
2039	AA111327	10^7 899-6										
2040	AA111328	10^7 899-6										
2041	AA111328	10^7 899-6										
2042	AA111328	10^7 899-6										
2043	AA111328	10^7 899-6										
		10^7 899-6										
2044	AA111329	10^7 3616										
2045	AA111329	10^7 3616										
2046	AA111329	10^7 3616										
2047	AA111329	10^7 3616										
2048	AA111330	10^7 3616										
2049	AA111330	10^7 3616										
2050	AA111330	10^7 3616										
2051	AA111330	10^7 3616										
		10^7 3616										
2052	AA111331	10^7 899-6+RT										
2053	AA111331	10^7 899-6+RT										
2054	AA111331	10^7 899-6+RT										
2055	AA111331	10^7 899-6+RT										
2056	AA111332	10^7 899-6+RT										
[illegible]	AA111332	10^7 899-6+RT										
[illegible]	AA111332	10^7 899-6+RT										
[illegible]	AA111332	10^7 899-6+RT										
		10^7 899-6+RT										

2060	AA111333	10^7 3616+RT										
2061	AA111333	10^7 3616+RT										
2062	AA111333	10^7 3616+RT										
2063	AA111333	10^7 3616+RT										
2067	AA111335	10^7 3616+RT										
2068	AA111335	10^7 3616+RT										
2069	AA111335	10^7 3616+RT										
2070	AA111335	10^7 3616+RT										
		10^7 3616+RT										

1/16/95

Greg's Mouse Log

Mouse #	Cage #	Group				Tumvol	weight				Tumvol	weight
1720	AA105283	10^7 899-6	21	15	11							
1710	AA105283	10^7 899-6	0	0	0							
1713	AA105283	10^7 899-6	0	0	0							
		10^7 899-6										
1697	AA104549	10^7 3616	18	16	10							
1708	AA104549	10^7 3616	0	0	0							
1709	AA104549	10^7 3616	3.5	3.5	1							
1723	AA104549	10^7 3616	2	2	1							
1875	AA108968	10^7 3616	17.5	19	8							
1876	AA108968	10^7 3616	20	20.5	13.5							
1877	AA108968	10^7 3616	24	18	17							
1878	AA108968	10^7 3616	20.5	17.5	13.5							
		10^7 3616										
1859	AA108972	RT Alone	9.5	10	3.5							
1860	AA108972	RT Alone	13	12.5	5.5							
1861	AA108972	RT Alone	11.5	12	5.5							
1862	AA108972	RT Alone	7	7	4							
1891	AA108964	RT Alone	6	6.5	3.5							
1892	AA108964	RT Alone	12.5	9	6							
1893	AA108964	RT Alone	9.5	8	4.5							
1894	AA108964	RT Alone	7	7	3.5							
		RT Alone										
1863	AA108971	10^7 3616+RT	9.5	8	3							
1864	AA108971	10^7 3616+RT	9	6.5	2							
1865	AA108971	10^7 3616+RT	5	3.5	3							
1866	AA108971	10^7 3616+RT	3	3	1							
1883	AA108966	10^7 3616+RT	3.5	2	1							
1884	AA108966	10^7 3616+RT	8.5	7	5.5							
Dead 1885	AA108966	10^7 3616+RT	~~2.5~~	~~3~~	~~1~~							
1886	AA108966	10^7 3616+RT	2.5	3	1							
		10^7 3616+RT										

1871	AA108969	10^7 899-6+RT	6	6	3.5							
1872	AA108969	10^7 899-6+RT	5.5	5	3							
1873	AA108969	10^7 899-6+RT	7	7	3.5							
1874	AA108969	10^7 899-6+RT	7.5	7.5	3							
1879	AA108967	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0							
1881	AA108967	10^7 899-6+RT	5.8	6	2.5							
1882	AA108967	10^7 899-6+RT	3	2.5	1							
		10^7 899-6+RT										
2032	AA111326	10^7 899-6	11	8	6							
2033	AA111326	10^7 899-6	13	9	6							
2034	AA111326	10^7 899-6	11.5	11	7							
2035	AA111326	10^7 899-6	12.5	8.5	8							
nets 2036	AA111327	10^7 899-6	11.5	9.5	6							
2037	AA111327	10^7 899-6	9.5	8	5.5							
2038	AA111327	10^7 899-6	13	11	8.5							
2039	AA111327	10^7 899-6	10.5	8.5	6							
2040	AA111328	10^7 899-6	13	13	8							
2041	AA111328	10^7 899-6	8	7.5	5.5							
2042	AA111328	10^7 899-6	12	14	11							
2043	AA111328	10^7 899-6	11	8.5	6							
		10^7 899-6										
2044	AA111329	10^7 3616	11	9	7							
2045	AA111329	10^7 3616	8	6.5	4.5							
2046	AA111329	10^7 3616	9	7.5	6							
2047	AA111329	10^7 3616	10.5	10.5	5							
2048	AA111330	10^7 3616	9	6	4.5							
2049	AA111330	10^7 3616	15	8	6.5							
2050	AA111330	10^7 3616	9	7.5	5.5							
2051	AA111330	10^7 3616	11	10	5.5							
		10^7 3616										
2052	AA111331	10^7 899-6+RT	10	7.5	6.5							
2053	AA111331	10^7 899-6+RT	6.5	8	6							
2054	AA111331	10^7 899-6+RT	9.5	7.5	4.5							
2055	AA111331	10^7 899-6+RT	14	9.5	6.5							
2056	AA111332	10^7 899-6+RT	10	7	4.5							
2057	AA111332	10^7 899-6+RT	7.5	7	5							
2058	AA111332	10^7 899-6+RT	10	12	5							
2059	AA111332	10^7 899-6+RT										
		10^7 899-6+RT										

2060	AA111333	10^7 3616+RT	9.5	6.5	4		21.6	895-6				
2061	AA111333	10^7 3616+RT	8	7	5.5		24.0					
2062	AA111333	10^7 3616+RT	6	7.5	4.5		18.9					
2063	AA111333	10^7 3616+RT	8	7	4.5		21.0					
2067	AA111335	10^7 3616+RT	7.5	6	5		21.3	3616				
2068	AA111335	10^7 3616+RT	10.5	8	6		21.0					
2069	AA111335	10^7 3616+RT	8	7.5	5.5		21.1					
2070	AA111335	10^7 3616+RT	10.5	6	5		17.8					
		10^7 3616+RT										

3/20

Greg's Mouse Log

	Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
	1720	AA105283	10^7 899-6	23	16	14		25.2					
	1710	AA105283	10^7 899-6	0	0	0		23.3					
	1713	AA105283	10^7 899-6	0	0	0		22.1					
	2052	AA111331	10^7 899-6	11	9.5	6		18.4					
	2053	AA111331	10^7 899-6	8	10.5	7		21.1					
	2054	AA111331	10^7 899-6	7	6	4.5		19.5					
14	2055	AA111331	10^7 899-6	10	12	7.5		17.4					
	2056	AA111332	10^7 899-6	9	7	5		23.4					
	2057	AA111332	10^7 899-6	7.5	6	4.5		21.8					
2	2058	AA111332	10^7 899-6	11	9.5	5.5		23.5					
	2060	AA111333	10^7 899-6	11	8.5	7		22.3					
	2061	AA111333	10^7 899-6	10	9	7		23.3					
	2062	AA111333	10^7 899-6	9	7.5	6.5		18.0					
	2063	AA111333	10^7 899-6	9.5	7.5	5.5		20.5					
			10^7 899-6										
	1697	AA104549	10^7 3616	18	15	11.5		23.4					
	1708	AA104549	10^7 3616	5	3	1		26.5					
SQC	1709	AA104549	10^7 3616	3	3	1		27.6					
	1723	AA104549	10^7 3616	0	0	0		26.1					
	~~1875~~	AA108968	10^7 3616	20.5	16.5	9		18.3	→SQC				
	1876	AA108968	10^7 3616	Dead									
	1877	AA108968	10^7 3616	26	19	18		26.3					
	1878	AA108968	10^7 3616	22	18.5	17		23.8					
15	~~2040~~	AA111328	10^7 3616	16	14	8.5		22.2	→	AA	111 335		
	2041	AA111328	10^7 3616	6	5.5	4		22.2					
12	~~2042~~	AA111328	10^7 3616	17	17	12		23.6	→	11			
	2043	AA111328	10^7 3616	10.5	9	5		24.0					
	2064	AA111334	10^7 3616	9.5	7	7		21.4					
4	2065	AA111334	10^7 3616	~~8~~13	~~8~~13	10		21.1					
	2066	AA111334	10^7 3616	11	7.5	6		16.5					
	2067	AA111334	10^7 3616	11	9.5	8		22.2					
			10^7 3616										
	2070			10.5	7	6		23.3					
	1859	AA108972	RT Alone	14.5	14	11		18.5					
	1860	AA108972	RT Alone	8	10	5.5		24.0					
	1861	AA108972	RT Alone	11.5	12	7.5		21.0					
	1862	AA108972	RT Alone	8	6.5	3.5		23.5					
	1891	AA108964	RT Alone	Dead									
	1892	AA108964	RT Alone	8	8	5	water	19.2					
	1893	AA108964	RT Alone	Dead									
	1894	AA108964	RT Alone	Dead									
			RT Alone										

	1863	AA108971	10^7 3616+RT	9	7	3		21.9		
	~~1864~~	AA108971	10^7 3616+RT	9	4.5	2.5		23.4		
	1865	AA108971	10^7 3616+RT	3.5	4.5	3		24.6		
	1866	AA108971	10^7 3616+RT	4	3	2		24.6		
	1883	AA108966	10^7 3616+RT	7	5	1.5		21.8		
	1884	AA108966	10^7 3616+RT	7	6.5	5		21.5		
	1886	AA108966	10^7 3616+RT	4	3	1		21.7		
15	2032	AA111326	10^7 3616+RT	10	6	4.5		20.9		
	2033	AA111326	10^7 3616+RT	11	8	5.5		22.2		
	~~2034~~	AA111326	10^7 3616+RT	14.5	13.5	10		18.2	→ 111	335
13	2035	AA111326	10^7 3616+RT	12	9	7.5		19.3		
	2036	AA111327	10^7 3616+RT	11	9	5.5		19.4		
	2037	AA111327	10^7 3616+RT	9	7	5.5		22.9		
3	~~2038~~	AA111327	10^7 3616+RT	13	11.5	7.5		17.4	→ 111	335
	2039	AA111327	10^7 3616+RT	9.5	8.5	6		19.0		
			10^7 3616+RT							
	1871	AA108969	10^7 899-6+RT	5	6	4		22.7		
	1872	AA108969	10^7 899-6+RT	7	6.5	3		23.1		
	1873	AA108969	10^7 899-6+RT	8.5	7	5		22.2		
	1874	AA108969	10^7 899-6+RT	7	6.5	3.5		23.3		
	1879	AA108967	10^7 899-6+RT	0	0	0		25.2		
	1880	AA108967	10^7 899-6+RT	4	4.5	1		21.5		
	1881	AA108967	10^7 899-6+RT	5	6.5	3		23.8		
	1882	AA108967	10^7 899-6+RT	4	3	1		28.0		
	2044	AA111329	10^7 899-6+RT	10	8	6		20.3		
	2045	AA111329	10^7 899-6+RT	6.5	5.5	4		23.4		
16	2046	AA111329	10^7 899-6+RT	9	7.5	5.5		20.7		
	2047	AA111329	10^7 899-6+RT	7	6.5	4		22.0		
	2048	AA111330	10^7 899-6+RT	8	5.5	4.5		20.2		
	2049	AA111330	10^7 899-6+RT	14.5	10	9		20.5		
	2050	AA111330	10^7 899-6+RT	6	8	4		21.2		
	2051	AA111330	10^7 899-6+RT	11	9	6.5		20		
			10^7 899-6+RT							
	2068	AA111~~335~~ 328	R 3616	8.5	7	5	111328	18.2		
	2069	AA111335	R 3616	8	8	7	104549	23.2		
	2070	AA111335	R 3616	10.5	7	6	"	23.3		
	2071	AA111336	R 3616	8	7.5	5.5	111328	23.0		
	2072	AA111336	I R3616+RT	7	6	4		23.8	111	326
	2073	AA111336	I R3616+RT	9.5	8	6.5		20.6	111	327
	2074	AA111336	I 3616+RT	9	6	7		17	108	966
	2075	AA111337	I 899-6	9	10	5		21.7	105	283
	2076	AA111337	I 899-6	8.5	8.5	5.5		23.8	111	332
	2077	AA111337	I 899-6 induction			inj	3/13		111	337
	2078	AA111337	I 899-6 induction			"			"	

3/21/95

In vivo TNF induction

PURPOSE - To see if TNFα induction production is induced in vivo with 20 Gy delivered 24° after injection of U87 hind limb tumors with R899-6 (10^7 PFU)

METHODS -
1. Implant subcut tumors in hind limb of nude mice
2. Grow to ~100 mm^3 in size
3. Inject with 2×10^7 PFU R899-6 (10 ul of stock)
4. 24 hours after injection randomize to +/- 20 Gy, and sacrifice at the following intervals:

Time	⊖ RT	⊕ RT
2° + 24°	4 mice	4 mice
→ 6° + 24°	"	"
→ 12° + 24°	"	"
→ 24° + 24°	"	"
→ 48° + 24°	"	"
→ 1 wk + 24°	"	"
→ 2 wk + 24°	"	"
3 wk + 24°	"	"

Need 64 mice

Infect W 9 AM
RT Th 9 AM

Sac @ ~~E~~ 3 PM
9 PM
F 9 AM

IN VIVO TNF INDUCTION

	1	2	3	4	5	6	7	8	9
A	1000		2161	6°	2185	12°	2169	24°	2181
B	500		2162	+ RT	2186	+	2170	+	2182
C	250		2163		2187	RT	2171	[redacted]	2183
D	125		2164		2188		2172		2184
E	62		2177		2141		2165		2173
F	31		2178	6°	2142	12°	2166	24°	2174
G	16		2179	no RT	2143	no	2167	[redacted]	2175
H	0		2180		2144	RT	2168	RT	2176

	10	11	12	13	14	15	16	17	18
A	48°	2153	1wk	2149	[redacted]				
B	+RT	2154	+	$\boxed{2150}$	[redacted]	1702	[redacted] control	2081	control 2
C		2155	RT	2151	[redacted]	1698	control	2145	2° [redacted]
D		2156		2152	[redacted]	1724	control	2146	2° RT
E						2042	[redacted]	2147	2° RT
F	48°	2157	[redacted] wk	2137	2wk	2034	[redacted]	2081	[illegible]
G	no RT	2158	[redacted] no	2138	no	2077	899-6	2145	
H		2159	RT	2139	RT	2078	89;%	2146	
		2160		2140		2080	control 1	[redacted]	

	19	
A	10^7 6°	+RT
B	10^7 6°	no RT
C	10^8 6°	+RT
D	10^8 6°	no RT
E	10^7 24°	+RT
F	10^7 24°	no RT
G	10^8 24°	+RT
H	10^8 24°	no RT

not all 1 supernatant assayed one was spilled

* for these sets, there was insufficient supernatant to fill the second well

649 x (C47 1.84)

PLATE #

OPERATOR: ______ O.D. LIMIT: [illegible] TIME: ______

WAVELENGTH: 405nm OPT 2 READ MODE: OPTICAL DENSITY AUTO MIX

NOTES: ______ CAL:

OPTICAL DENSITY

	1	2	3	4	5	6	7	8	9	10	11	12
			6° +RT		12° +RT		24° +RT		48° +RT		1wk +RT	
A	[illegible]	0.282	[illegible]	0.704	0.820	0.825	[illegible]	0.358	0.909	0.799	0.317	[illegible]
B	0.268	0.203	0.654	0.613	0.405	0.361	0.731	0.708	1.348	1.482	0.365	0.327
C	0.232	0.175	0.320	0.287	0.300	0.279	0.621	[redacted]	0.922	0.843	0.453	0.331
D	[illegible]	0.105	0.457	0.391	[illegible]	0.825	[illegible]	1.280	0.423	0.407	0.318	[illegible]
			6° ⊖RT		12° ⊖RT		24° no RT		48° ⊖RT		1wk ⊖ RT	
E	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.737	0.706	[illegible]	[redacted]
F	[illegible]	0.078	[illegible]	[illegible]	0.702	[illegible]	[illegible]	[redacted]	[illegible]	0.774	[illegible]	[redacted]
G	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.730	[illegible]	[illegible]	[illegible]	[illegible]
H	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

PLATE #

O.D. LIMIT:

WAVELENGTH: [illegible] READ MODE: [illegible] AUTO MIX

▬ = invalid data

CAL:

OPTICAL DENSITY

	1 (13)	2 (14)	3 (15)	4 (16)	5 (17)	6 (18)	7 (19)	8 (20)	9	10	11	12
	2wk + RT						10^7					
A	[illegible]	[redacted]	[illegible]	[redacted]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
B	[redacted]	[redacted]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
							10^8					
C	[illegible]	[redacted]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
D	[illegible]	[redacted]	[illegible]	[redacted]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	2wk no RT						10^7					
E	[illegible]	[illegible]	[illegible]	[redacted]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
F	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
							10^8					
G	[illegible]	[illegible]	[illegible]	[illegible]	[redacted]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

INDUCTION-'in vivo							
Mouse	Treatment	Rx Date	Sac Date	Size	Tumor Wt	Vol Buffer	[TNF]pg/ml
1702	Control	None	2/16/95	63	0.06	.6	0.37
~~1698~~	Control	None	2/9/95	67	~~0.07~~ 2.0	1.0	1.03
1724	Control	None	2/16/95	2520	~~2.5~~ 0.07	.6	0.42
2042	3616	3/13/95	4/3/95	528	0.6	.6	0.33
2034	3616+20+25	3/13/95	4/3/95	644	0.7	.6	0.41
2077	899-6	3/13/95	4/3/95	2090	2.9	1.0	~~0.44~~ 0.89
2078	899-6	3/13/95	4/3/95	6186	4.5	1.0	0.44
2080	Control 1	None	4/3/95	938	0.9	.6	0.52
2081	Control 2	None	4/3/95	1124	1	1.0	1.31
2145	2 hrs + RT	4/26/95	4/27/95		0.51	.6	0.57
2146	2 hrs + RT	4/26/95	4/27/95		0.39	.6	0.49
2147	2 hrs + RT	4/26/95	4/27/95		0.06	.6	0.40

8

52
.6
31.2
8
~~39~~ 249.2

40ml 150mM NaCl
400ul TRIS 1M pH7.5
400ul EDTA 0.5m pH7.5

protease inhibitors
/ DTT 1mM 40ul
/ PMSF 100mM 20ul
leupeptin 5ug 4ul
2 aprotinin 10ug 8ul

26 + 8 = 34 ml
2.52

76
2
8|152 wells

19 rows
8
152

2

THE UNIVERSITY OF CHICAGO ANIMAL RESOURCES CENTER ANIMAL PROCUREMENT REQUEST (#315B)

REQ NO 18784

ARC USE ONLY

P.O. # ____

ORDER DATE ____

REF. # ____

CONTACT ____

EST AMT ____

SCHED DEL ____

SPECIAL ROUTING

NON COM VENDOR ____

FLAGGED BY PROTOCOL ____

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ____ SHIPMENTS ON A ____ BASIS

REQUEST BY: Greg Sibley, MD DATE: 3/21/95

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: [signature]

FAS ACCOUNT: 2-73731-5100 P.I.: Hallahan

VENDOR: FCRI PROTOCOL: 58671

REQUESTED DELIVERY DATE: 3/27/95 PHONE: 2-0294

SPECIES: Mouse QUANTITY: 80

STRAIN: Athymic Nude SEX: M F EITHER

WEIGHT/AGE: 5-6 weeks ALTERNATE WEIGHT/AGE: ____

(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS: ____

HOUSE AT: ____ CARLSON ____ WYLER X CLSC ____ FMI ____ OTHER

3/23

1863	AA108971	10^7 3616+RT	10.5	8	3
1864	AA108971	10^7 3616+RT	6	8	3.5
1865	AA108971	10^7 3616+RT	3	3	2
1866	AA108971	10^7 3616+RT	3	7.5	1.5
1883	AA108966	10^7 3616+RT	4.5	4	1
1884	AA108966	10^7 3616+RT	7.5	6	5
1886	AA108966	10^7 3616+RT	6	4	3
2074	AA108966	10^7 3616+RT	6.5	5	7
2032	AA111326	10^7 3616+RT	10	6	5.5
2033	AA111326	10^7 3616+RT	12	9	5.5
2035	AA111326	10^7 3616+RT	8.5	7.5	6
2072	AA111326	10^7 3616+RT	13	9.5	8
2036	AA111327	10^7 3616+RT	12	9	6
2037	AA111327	10^7 3616+RT	8.5	7	6
2039	AA111327	10^7 3616+RT	10	9	5
2073	AA111327	10^7 3616+RT	12.5	11.5	9
		10^7 3616+RT			
1871	AA108969	10^7 899-6+RT	6.5	7	4
1872	AA108969	10^7 899-6+RT	4.5	6.5	2.5
1873	AA108969	10^7 899-6+RT	8	8	3.5
1874	AA108969	10^7 899-6+RT	7	6	3
1879	AA108967	10^7 899-6+RT	0	0	0
1880	AA108967	10^7 899-6+RT	3.5	3	1
1881	AA108967	10^7 899-6+RT	6	4.5	2
1882	AA108967	10^7 899-6+RT	3	2.5	1
2044	AA111329	10^7 899-6+RT	9.5	8	5
2045	AA111329	10^7 899-6+RT	5.5	5	3
2046	AA111329	10^7 899-6+RT	9.5	8	5
2047	AA111329	10^7 899-6+RT	6	6	3.5
2048	AA111330	10^7 899-6+RT	7.5	6	4.5
2049	AA111330	10^7 899-6+RT	15	10	8
2050	AA111330	10^7 899-6+RT	7	6.5	4
2051	AA111330	10^7 899-6+RT	11	13	5
		10^7 899-6+RT			

The University of Chicago Departmental Purchase Order

Z890581

THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

Sarstedt
St James Church Rd
Newton, N.C. 28658
City State Zip Code

Payment Terms ______ Delivery charge? ☐ Yes ☒ No

Telephone No. 800-257-5101 FAX No. ______

Ship To

THE UNIVERSITY OF CHICAGO Dept. Code: ______
Radiation Oncology MC-0085
Department
~~6-03~~ Greg Sibley 6-03
Attn: Room
CHGO IL 60637
City State Zip Code

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? ☐ No ☒ Yes
Mary 3/24/95
Order placed with (name) Date
Greg Sibley 800-257-5101
Order placed by (name) Phone #

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1000	65816 Dome stopper	19.22	
1	1000	55.518 13 ml centrifuge tubes	57.92	
			77.14	
			ORDER TOTAL	

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00

BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

7.

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
			3/27/94									
1720	AA105283	10^7 899-6	25	17.5	14		26.5					
1710	AA105283	10^7 899-6	0	0	0		22.7					
2075	AA105283	10^7 899-6	10	9	3		20.7					
1713	AA105283	10^7 899-6	0	0	0		21.2					
2052	AA111331	10^7 899-6	14.5	12	8		20.3					
2053	AA111331	10^7 899-6	12	15.5	8		22.7					
2054	AA111331	10^7 899-6	19	15	6		19.7					
2055	AA111331	10^7 899-6	11	7	3.5		21.9					
2056	AA111332	10^7 899-6	12	15	7		24.5					
2057	AA111332	10^7 899-6	6	6	3		21.4					
2058	AA111332	10^7 899-6	12.5	11	6		23.9					
2076	AA111332	10^7 899-6	9.5	11	7		24.6					
2060	AA111333	10^7 899-6	~~19~~	~~17.5~~	~~9.5~~		~~25.3~~	17.5	12.5	8		23.7
2061	AA111333	10^7 899-6	11	10	5		22.6					
2062	AA111333	10^7 899-6	8	6	4		16.9					
2063	AA111333	10^7 899-6	9	8	6.5		21.0					
		10^7 899-6										
1697	AA104549	10^7 3616	19	17.5	9.5		25.3					
1708	AA104549	10^7 3616	0	0	0		21.9					
1709	AA104549	10^7 3616	0	0	0		27.3					
1723	AA104549	10^7 3616	0	0	0		26.3					
1877	AA108968	10^7 3616										
1878	AA108968	10^7 3616										
2041	AA111328	10^7 3616	6	5	3		22.3					
2043	AA111328	10^7 3616	13.5	12	7		25					
2064	AA111334	10^7 3616	8	6.5	4		23.1					
2065	AA111334	10^7 3616	16	16	9		23.3					
2066	AA111334	10^7 3616	12	12	6		23.2					
2067	AA111334	10^7 3616	8.5	6	3.5		14.5					
2068		10^7 3616	7	6	5		15.8					
2069		10^7 3616	10	10	7.5		23.2					
2070		10^7 3616	11	8	5		23.4					
2071		10^7 3616	11	10	5		24.0					
		10^7 3616										
1859	AA108972	RT Alone	9.5	10	3.5		23.9					
~~1860~~	AA108972	RT Alone	3/27/95									
1861	AA108972	RT Alone	12	13.5	7		21.5					
1862	AA108972	RT Alone	8	7	3		25.3					
1892	AA108964	RT Alone	7.5	8	5		22.8					
		RT Alone										
			~~12~~	~~13.5~~	~~7~~		~~21.5~~					

1863	AA108971	10^7 3616+RT	10	7	2.5		22.9					
1864	AA108971	10^7 3616+RT	7.5	5	3		24.1					
1865	AA108971	10^7 3616+RT	3.5	3	1		24.9					
1866	AA108971	10^7 3616+RT	3	3	1		24.9					
1883	AA108966	10^7 3616+RT	0	0	0		22					
1884	AA108966	10^7 3616+RT	5.5	5.5	3		20.6					
1886	AA108966	10^7 3616+RT	11.5	10	7		14.8					
2074	AA108966	10^7 3616+RT	4	4	1		22					
2032	AA111326	10^7 3616+RT	11.5	7.5	5		20.2					
2033	AA111326	10^7 3616+RT	11.5	9	6		21.6					
2035	AA111326	10^7 3616+RT	5.5	5.5	3		27.7					
2072	AA111326	10^7 3616+RT	13	10	6		18.8					
2036	AA111327	10^7 3616+RT	12	9	4		20.4					
2037	AA111327	10^7 3616+RT	6	8	4		23.6					
2039	AA111327	10^7 3616+RT	11	8	7.5		19.3					
2073	AA111327	10^7 3616+RT	8.5	10	5		17.9					
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	7	7	3.5		23.3					
1872	AA108969	10^7 899-6+RT	4.5	6	3		22.4					
1873	AA108969	10^7 899-6+RT	7.5	8	4		22.4					
1874	AA108969	10^7 899-6+RT	6.5	5	2.5		24.8					
1879	AA108967	10^7 899-6+RT	0	0	0		26.0					
1880	AA108967	10^7 899-6+RT	0	0	0		20.7					
1881	AA108967	10^7 899-6+RT	0	0	0		24.4					
1882	AA108967	10^7 899-6+RT	4	3	1		22.1					
2044	AA111329	10^7 899-6+RT	9	7.5	5.5		20.9					
2045	AA111329	10^7 899-6+RT	7	5	3		24.5					
2046	AA111329	10^7 899-6+RT	8.5	7	5		22.7					
2047	AA111329	10^7 899-6+RT	5	5	5		21.2					
2048	AA111330	10^7 899-6+RT	5.5	8	3.5		19.2					
2049	AA111330	10^7 899-6+RT	15	10.5	5		21.8					
2050	AA111330	10^7 899-6+RT	6	6	4		21.4					
2051	AA111330	10^7 899-6+RT	17.5	11	4.5		20.5					
		10^7 899-6+RT										

3/27/85

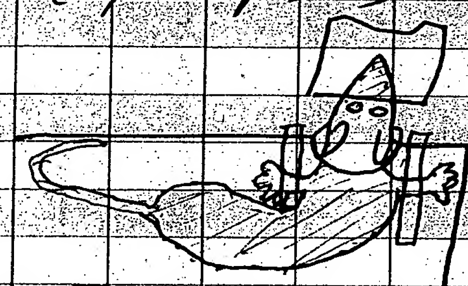
TOLERANCE OF NUDE MICE TO WHOLE BRAIN RT

PURPOSE - In preparation for stereotactic brain protocol, need to determine maximum tolerated whole brain dose

PROCEDURE -

① Anesthetize mice c̄ Ketamine / Xylazine 0.4 ml...

② Tape front legs of mice with lead protruding through lead shield & piece of lead over nose (eyes exposed)



③ Irradiate c̄ Maxitron: Plexiglass-to-coll dist = 8cm, 30 mA 150 KVp (see TLD measurements next page)

TLD @ surface of skull = 450-500 cGy
TLD under head = 325 cGy } 500 c... intended

④ Pilot experiment = 3 mice

mouse #1 = 1000 x 5 = 5000 cGy
#2 = 500 x 6 = 3000 cGy
#3 = [illegible]00 x 8 = 4000 cGy

RESULTS -

	RT dates start	end	Status
mouse #1	3/27	3/31	died 4/5
mouse #2	3/27	4/3	
mouse #3	3/27	4/5	

3-27-96 TLD exposure Measurement on Mice for

Dr. Smith, & Hyland Chromal .035" TLDs

100 KVp
30 mA
1 mm Al Filter

Calibration TLDs
500 cGy at above beam quality

11.38 μC
11.29
12.33
11.38

11.59 ± .60

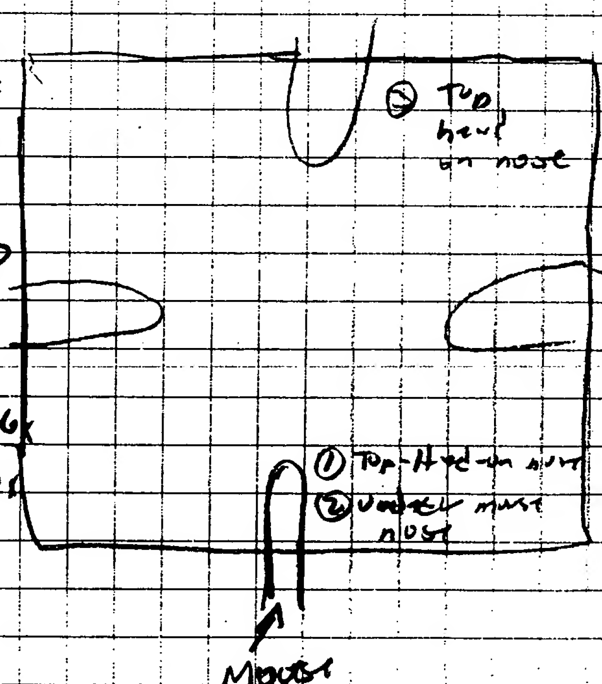
$$\left(\frac{TL}{D}\right)_{100KVp} = \frac{11.59\,\mu C}{5.00\,Gy} = 2.32\,\frac{\mu C}{Gy}$$

TLDs on Mice

① 11.54 μC 497 cGy
10.42 466 cGy ⟨482 cGy⟩

② cGy
6.81 μC 294
8.27 356
⟨325 cGy⟩

③ 11.18 482 cGy
9.60 414 cGy
⟨448⟩



The University of Chicago Departmental Purchase Order

Purchase Order Number **Z890593**
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

Fisher Scientific
1600 W Glenlake Ave
Itasca, IL 60143
City | State | Zip Code

Payment Terms ______ Delivery charge? ☐ Yes ☐ No

Telephone No. 2-3328 FAX No. ______

Ship To

THE UNIVERSITY OF CHICAGO Dept. Code: ______

U of C Rad Onc
Department

Greg Sibley Room G-08
Attn:

5830 S. Ellis MC 0085

Chgo IL 60637
City | State | Zip Code

Authorized Signature ______ Payroll No. ______

Print Name ______ Ext. ______

Account Code ______ Date ______

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Order placed by phone? ☐ No ☒ Yes

Cindy — Order placed with (name) — 3/31/95 Date

Greg Sibley — Order placed by (name) — 2-0294 Phone #

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
4	ea	Hamilton Syringes 10 ul 14-824-3	23.04	92.16
2	Box	15 ml polypropylene tubes 14-959-70C	$ 83.40	166.80
				~~266.96~~
				258.96
			ORDER TOTAL	

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00

BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

4/3/95

INDUCTION- in vivo					
Mouse	Treatment	Date	Size	Sac Date	Tumor wt
1702	Control		63	2/16/95	
1698	Control		67.375	2/9/95	
1724	Control		2520	2/16/95	
2040	3616	3/13/95	died 4/3		
2042	3616	3/13/95	12·11·8	4/3	0.6
2034	3616+20+25	3/13/95	13·11·9	"	0.7
~~2038~~	~~3616+20+25~~	~~3/13/95~~			
ß 2077	899-6	3/13/95	4/3	22·19·10	2.9 gm
RR 2078	899-6	3/13/95	"	27·23.5·15.5	4.5
~~2079~~	~~899-6~~	~~3/13/~~95	died 4/3		
2080 control 1		15·12.5·10	"		0.9 gm
2081 2		14.5·15.5·10	"		1.0 gm

4/3/95

Greg's Mouse Log

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
~~1720~~	AA105283	10^7 899-6	20.5	28	16.5		29.3					
1710	AA105283	10^7 899-6	0	0	0		23.6					
2075	AA105283	10^7 899-6	12	12	7		23.4					
1713	AA105283	10^7 899-6	0	0	0		21.6					
2052	AA111331	10^7 899-6	16.5	14.5	12		21.4					
2053	AA111331	10^7 899-6	21	15	11.5		24.1					
2054	AA111331	10^7 899-6	16	12	7.5		22.0					
~~2055~~	AA111331	10^7 899-6	23.5	21	10.5		22.75	ecked				
2056	AA111332	10^7 899-6	20	18	11		25.4					
2057	AA111332	10^7 899-6	9.5	8	6		21.8					
2058	AA111332	10^7 899-6	19	20	8.5		25.9					
2076	AA111332	10^7 899-6	15	16.5	9		27.5					
2060	AA111333	10^7 899-6	19.5	18	13		25.8					
2061	AA111333	10^7 899-6	20	16	11.5		26.2					
2062	AA111333	10^7 899-6	13.5	8	8.5		17.2					
2063	AA111333	10^7 899-6	17.5	13	10.5		23.5					
		10^7 899-6										
1697	AA104549	10^7 3616	18.5	18.5	13		25.2					
1708	AA104549	10^7 3616	0	0	0		21.4					
1709	AA104549	10^7 3616	0	0	6		25.7					
1723	AA104549	10^7 3616	0	0	0		26.0					
2069	AA108968	10^7 3616	12	15	8		24.8					
2070	AA108968	10^7 3616	13	11	6		26.2					
2041	AA111328	10^7 3616	8	8	5.5		23.6					
2043	AA111328	10^7 3616	22	17	11.5		26.5					
2068	AA111328	10^7 3616	5	4.5	3		19.9					
2071	AA111328	10^7 3616	15	12.5	8		26.3					
2064	AA111334	10^7 3616	8.5	8	5		24.1					
~~2065~~	AA111334	10^7 3616	23	24	14		25.8					
2066	AA111334	10^7 3616	16	15.5	6.5		25.2					
~~2067~~	AA111334	10^7 3616	8	6	4.5		12.4					
90		10^7 3616										
1859	AA108972	RT Alone	9	9	4		25.5					
1861	AA108972	RT Alone	12	14.5	8		20.5					
1862	AA108972	RT Alone	6.5	8	5		24.9					
1892	AA108964	RT Alone	7	6.5	3.5		22.7					
		RT Alone										

4/3/95

1863	AA108971	10^7 3616+RT	8.5	7	3		23.4					
1864	AA108971	10^7 3616+RT	6.5	4	4		24.4					
1865	AA108971	10^7 3616+RT	2	2	1		24.1					
1866	AA108971	10^7 3616+RT	3.5	3	1		25.0					
1883	AA108966	10^7 3616+RT	0	0	0		22.2					
1884	AA108966	10^7 3616+RT	4.5	4	2		22.7					
1886	AA108966	10^7 3616+RT	10	10	4		14.6					
2074	AA108966	10^7 3616+RT	0	0	0		22.3					
2032	AA111326	10^7 3616+RT	7	5.5	5.5		19.5					
2033	AA111326	10^7 3616+RT	11.5	8	6		21.2					
2035	AA111326	10^7 3616+RT	10.5	8.5	7		18.3					
2072	AA111326	10^7 3616+RT	4	5	2.5		22.1					
2036	AA111327	10^7 3616+RT	11	8.5	5		21.0					
2037	AA111327	10^7 3616+RT	6.5	5	3.5		24.5					
2039	AA111327	10^7 3616+RT	12.5	11	6		18.2					
2073	AA111327	10^7 3616+RT	7.5	7.5	2.5		18.9					
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	6	6	3		24.5					
1872	AA108969	10^7 899-6+RT	6	4.5	3.5		23.4					
1873	AA108969	10^7 899-6+RT	7	8.5	5		24.1					
1874	AA108969	10^7 899-6+RT	5.5	3.5	2		25.8					
1879	AA108967	10^7 899-6+RT	0	0	0		26.4					
1880	AA108967	10^7 899-6+RT	0	0	0		22.1					
1881	AA108967	10^7 899-6+RT	0	0	0		23.4					
1882	AA108967	10^7 899-6+RT	0	0	0		24.7					
2044	AA111329	10^7 899-6+RT	6.5	7.5	4		23.2					
2045	AA111329	10^7 899-6+RT	3	3.5	1.5		25.2					
2046	AA111329	10^7 899-6+RT	6.5	6	4		24.2					
2047	AA111329	10^7 899-6+RT	0	0	0		22.4					
2048	AA111330	10^7 899-6+RT	4.5	3.5	2.5		20.0					
2049	AA111330	10^7 899-6+RT	12.5	11	7		23.4					
2050	AA111330	10^7 899-6+RT	6	5	4		23.5					
2051	AA111330	10^7 899-6+RT	9	6.5	4		23.2					
		10^7 899-6+RT										

4/6/95

1863	AA108971	10^7 3616+RT	8.5	7	4			
1864	AA108971	10^7 3616+RT	8.5	6	5			
1865	AA108971	10^7 3616+RT	3	3	1			
1866	AA108971	10^7 3616+RT	~~7.5~~	~~3~~	~~1~~	0	0	0
1883	AA108966	10^7 3616+RT	5	4	1			
1884	AA108966	10^7 3616+RT	3.5	3	2.5			
1886	AA108966	10^7 3616+RT	0	0	0			
207[illegible]	AA108966	10^7 3616+RT	9.5	10.5	4.5			
2032	AA111326	10^7 3616+RT	6.5	5	4.5			
2033	AA111326	10^7 3616+RT	11	8	6			
2035	AA111326	10^7 3616+RT	11	8.5	6.5			
2072	AA111326	10^7 3616+RT	6	4	1			
2036	AA111327	10^7 3616+RT	10	9.5	4.5			
2037	AA111327	10^7 3616+RT	4.5	6	2.5			
2039	AA111327	10^7 3616+RT	10	12	7.5			
2073	AA111327	10^7 3616+RT	7	6.5	3			
		10^7 3616+RT						
1871	AA108969	10^7 899-6+RT	6.5	6	3			
1872	AA108969	10^7 899-6+RT	~~6~~ 4	~~4~~ 6	~~4~~ 3.5			
1873	AA108969	10^7 899-6+RT	7	7.5	4.5			
1874	AA108969	10^7 899-6+RT	3	3.5	1.5			
1879	AA108967	10^7 899-6+RT	0	0	0			
1880	AA108967	10^7 899-6+RT	4	5.5	1.5			
1881	AA108967	10^7 899-6+RT	4	3	1			
1882	AA108967	10^7 899-6+RT	0	0	0			
2044	AA111329	10^7 899-6+RT	~~7.5~~	~~6~~	~~3.5~~	6	6.5	4
2045	AA111329	10^7 899-6+RT	3.5	3	1			
2046	AA111329	10^7 899-6+RT	2.5	6	3.5			
2047	AA111329	10^7 899-6+RT	3	3	1			
2048	AA111330	10^7 899-6+RT	4	4	3			
2049	AA111330	10^7 899-6+RT	13	11	7			
2050	AA111330	10^7 899-6+RT	3	5	2.5	edema		
2051	AA111330	10^7 899-6+RT	6.5	9	4			
		10^7 899-6+RT						
2082	AA111338	S brain RT	1000 x 5 died					
2083	AA111339	S "	500 x 8					
2084	AA111339	S "	500 x 6					
2085	AA111339	S stereotactic						
2086	AA111339	S "						
2087	AA111340	S "						
2088	AA111340	S "						
2089	AA111340	S						
2090	AA111340	S						

4/6/95

Greg's Mouse Log

	Mouse #	Cage #	Group						
	1710	AA105283	10^7 899-6	0	0	0			
	2075	AA105283	10^7 899-6	16	14	8.5			
	1713	AA105283	10^7 899-6	0	0	0			
ic	~~2052~~	AA111331	10^7 899-6	23	17.5	13			
	~~2053~~	AA111331	10^7 899-6	18.5	17	13	sacked 4/6		
	2054	AA111331	10^7 899-6	28	14.5	13	16.5	12	8.5
	~~2056~~	AA111332	10^7 899-6	21.5	15.5	12			
33←	2057	AA111332	10^7 899-6	9.5	21	6.5			
	~~2058~~	AA111332	10^7 899-6	19.5	21.5	10.5			
33←	2076	AA111332	10^7 899-6	17	18.5	9.5			
	~~2061~~	AA111333	10^7 899-6	23	18.5	12.5			
	2062	AA111333	10^7 899-6	15.5	11	10			
	2063	AA111333	10^7 899-6	20.5	16	11.5			
			10^7 899-6						
SAC	~~1697~~	AA104549	10^7 3616	18.5	19	12			
	1708	AA104549	10^7 3616	0	0	0			
	1709	AA104549	10^7 3616	0	0	0			
	1723	AA104549	10^7 3616	0	0	0			
334←	2069	AA108968	10^7 3616	13.5	17	~~10~~ 7.5	remeasure		
334	2070	AA108968	10^7 3616	13	16	17.5			
	2041	AA111328	10^7 3616	10.5	9.5	6.5			
	2068	AA111328	10^7 3616	4.5	4	2.5			
	2071	AA111328	10^7 3616	18	16.5	10			
	2064	AA111334	10^7 3616	9	10	7			
	2066	AA111334	10^7 3616	20	17	9			
			10^7 3616						
	1859	AA108972	RT Alone	10	10	4			
	1861	AA108972	RT Alone	12	13	10.5			
	1862	AA108972	RT Alone	6.5	7	4			
	1892	AA[illegible]08964	RT Alone	7.5	7.5	3.5			
			RT Alone						

[illegible]

~~9 10 5.5 14.4~~ 4/10/95

1863	AA108971	10^7 3616+RT	9	6.5	4		27.2					
1864	AA108971	10^7 3616+RT	9	8.5	7		27.0					
1865	AA108971	10^7 3616+RT	2	2	1		27.1					
1866	AA108971	10^7 3616+RT	0	0	0		25.8					
1883	AA108966	10^7 3616+RT	0	0	0		21.8					
1884	AA108966	10^7 3616+RT	2	2	2		20.9					
1886	AA108966	10^7 3616+RT	0	0	0		21.8					
2074	AA108966	10^7 3616+RT	9	10	5.5		14.4					
2032	AA111326	10^7 3616+RT	9.5	7	5.5		20.9					
2033	AA111326	10^7 3616+RT	6	5	4		18.2					
2035	AA111326	10^7 3616+RT	13.5	12	6		18.5					
2072	AA111326	10^7 3616+RT	0	0	0		22.7					
2036	AA111327	10^7 3616+RT	10.5	9	4		20.7					
2037	AA111327	10^7 3616+RT	5	4	2.5		23.7					
2039	AA111327	10^7 3616+RT	8	7	4.5		18.6					
2073	AA111327	10^7 3616+RT	10	9	4.5		18.2					
		10^7 3616+RT										
1871	AA108969	10^7 899-6+RT	6	5.5	2.5		19.4					
1872	AA108969	10^7 899-6+RT	4.5	6	4		23.5					
1873	AA108969	10^7 899-6+RT	7	7.5	2.5		24.0					
1874	AA108969	10^7 899-6+RT	3	2	1		26.1					
1879	AA108967	10^7 899-6+RT	0	0	0		27.2					
1880	AA108967	10^7 899-6+RT	0	0	0		20.7					
1881	AA108967	10^7 899-6+RT	0	0	0		25.2					
1882	AA108967	10^7 899-6+RT	0	0	0		23.7					
2044	AA111329	10^7 899-6+RT	4.5	5	3.5		23.2					
2045	AA111329	10^7 899-6+RT	0	0	0		25.8					
2046	AA111329	10^7 899-6+RT	6.5	5.5	4		23.7					
2047	AA111329	10^7 899-6+RT	0	0	0		22.5					
2048	AA111330	10^7 899-6+RT	3.5	3	2.5		18.0					
2049	AA111330	10^7 899-6+RT	6.5	9	4.5		22.0					
2050	AA111330	10^7 899-6+RT	3	4	2.5		22.4					
2051	AA111330	10^7 899-6+RT	12.5	11	8		23.0					
		10^7 899-6+RT										
2082	AA111338	S										
2083	AA111339	S										
2084	AA111339	S										
2085	AA111339	S										
2086	AA111339	S										
2087	AA111340	S										
2088	AA111340	S										
2089	AA111340	S										
2090	AA111340	S										

April 12, 1995

Jim Linsley
Animal Resource Center
Room P-110

Dear Jim,

We placed our mice on Bactrim in Cummings Rm #1053 (on 4/12/95) due to the appearance of declining health and a number of deaths after tumor implantation. I apologize for not making you aware of this. Please continue Bactrim therapy for 1 week (until 4/18/95).

The mice placed on antibiotics include all mice with HALLAHAN/SIBLEY on the card (20 cages).

Cage #'s:

Sorry for the confusion.

Sincerely,

Gregory S. Sibley, M.D.

4/13

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0							
2075	AA105283	10^7 899-6	21.5	19.5	9							
1713	AA105283	10^7 899-6	0	0	0							
2054	AA111331	10^7 899-6	11	14	4.5							
2057	AA111332	10^7 899-6	13	10	7							
1709	AA104549	10^7 3616	0	0	0							
1723	AA104549	10^7 3616	0	0	0							
~~2069~~	AA108968	10^7 3616	23	20	13.5							
~~2070~~	AA108968	10^7 3616	23	19	11							
2064	AA111334	10^7 3616	15	14	7.5							
2041	"	10^7 3616	12	11	9							
1859	AA108972	RT Alone	11	10	6							
1861	AA108972	RT Alone	13.5	12.5	8							
1862	AA108972	RT Alone	6	6	3.5							
1892	AA108964	RT Alone	6	6	3.5							
1863	AA108971	10^7 3616+RT	8	6	4							
1864	AA108971	10^7 3616+RT	12	10	9							
1865	AA108971	10^7 3616+RT	0	0	0							
1866	AA108971	10^7 3616+RT	0	0	0							
1883	AA108966	10^7 3616+RT	0	0	0							
1884	AA108966	10^7 3616+RT	8	3.5	2							
1886	AA108966	10^7 3616+RT	0	0	0							
2074	AA108966	10^7 3616+RT	9	10	5							
2032	AA111326	10^7 3616+RT	6	5	5							
2033	AA111326	10^7 3616+RT	9	7	3.5							
2035	AA111326	10^7 3616+RT	9	7	6							
2072	AA111326	10^7 3616+RT	0	0	0							
2036	AA111327	10^7 3616+RT	10	9	4							
2037	AA111327	10^7 3616+RT	5	3.5	2.5							
2039	AA111327	10^7 3616+RT	7	8	3.5							
2073	AA111327	10^7 3616+RT	9.5	9	5							
1871	AA108969	10^7 899-6+RT	6.5	6.5	4							
1872	AA108969	10^7 899-6+RT	4	5	3.5							
1873	AA108969	10^7 899-6+RT	7	7	4.5							
1874	AA108969	10^7 899-6+RT	0	0	0							
1879	AA108967	10^7 899-6+RT	0		0							
1880	AA108967	10^7 899-6+RT	0		0							
1881	AA108967	10^7 899-6+RT	0		0							
1882	AA108967	10^7 899-6+RT	0		0							
2044	AA111329	10^7 899-6+RT	4.5	4.[illegible]	3.5							
2045	AA111329	10^7 899-6+RT	0	0	0							
2046	AA111329	10^7 899-6+RT	6.5	6	3							
2047	AA111329	10^7 899-6+RT	0	0	0							
2048	AA111330	10^7 899-6+RT	3	3.5	2							
2049	AA111330	10^7 899-6+RT	14	12	10							
2050	AA111330	10^7 899-6+RT	3	2.5	1							
2051	AA111330	10^7 899-6+RT	9	6.5	4.5							

4/17/95

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0		23.0					
~~2075~~	~~AA105283~~	~~10^7 899-6~~	21	18	6.5		21.7					
1713	AA105283	10^7 899-6	0	0	0		21.6					
2054	AA111331	10^7 899-6	11	15	4		19.2					
2057	AA111332	10^7 899-6	14.5	12	8.5		21.1					
1709	AA104549	10^7 3616	0	0	0		23.9					
1723	AA104549	10^7 3616	0	0	0		24.4					
2069	AA108968	10^7 3616										
2070	AA108968	10^7 3616										
2064	AA111334	10^7 3616	15.5	14	8		23.4					
2041		3616	13.5	12	9		21.9					
1859	AA108972	RT Alone	11.5	10	5		26.1					
1861	AA108972	RT Alone	6.5	6	4.5		24.9					
1862	AA108972	RT Alone	14	14	7.5		22.5					
1892	AA108964	RT Alone	6.5	6.5	3.5		22.8					
1863	AA108971	10^7 3616+RT	8	5	3		24.5					
1864	AA108971	10^7 3616+RT	12	11	8.5		25.6					
1865	AA108971	10^7 3616+RT	0	0	0		26.4					
1866	AA108971	10^7 3616+RT	0	0	0		25.8					
1883	AA108966	10^7 3616+RT	0	0	0		22.0					
1884	AA108966	10^7 3616+RT	3	3	2		22.3					
1886	AA108966	10^7 3616+RT	10	9	4.5		16.4					
2074	AA108966	10^7 3616+RT	0	0	0		21.5					
2032	AA111326	10^7 3616+RT	6	5	5		19.7					
2033	AA111326	10^7 3616+RT	6	5.5	3.5		22.4					
2035	AA111326	10^7 3616+RT	8	6	4		19.3					
2072	AA111326	10^7 3616+RT	0	0	0		22.7					
2036	AA111327	10^7 3616+RT	10	9	4		22.5					
2037	AA111327	10^7 3616+RT	4	5	2.5		24.0					
2039	AA111327	10^7 3616+RT	8	8	4		18.4					
2073	AA111327	10^7 3616+RT	8	9	5		19.4					
1871	AA108969	10^7 899-6+RT	5	5	2		25.4					
1872	AA108969	10^7 899-6+RT	6.5	5.5	3		22.6					
1873	AA108969	10^7 899-6+RT	7	7.5	5		23.3					
1874	AA108969	10^7 899-6+RT	0	0	0		26.1					
1879	AA108967	10^7 899-6+RT	0	0	0		28.7					
1880	AA108967	10^7 899-6+RT	0	0	0		21.9					
1881	AA108967	10^7 899-6+RT	0	0	0		25.6					
1882	AA108967	10^7 899-6+RT	0	0	0		23.6					
2044	AA111329	10^7 899-6+RT	4	4	3.5		22.8					
2045	AA111329	10^7 899-6+RT	0	0	0		25.6					
2046	AA111329	10^7 899-6+RT	6.5	6	3		23.7					
2047	AA111329	10^7 899-6+RT	0	0	0		21.7					
2048	AA111330	10^7 899-6+RT	4	3	2		18.3					
2049	AA111330	10^7 899-6+RT	14	13	6		22.6					
2050	AA111330	10^7 899-6+RT	3	3	2		22.7					
2051	AA111330	10^7 899-6+RT	8	10	3.5		21.7					

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Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	ϕ									
~~2075~~	~~AA105283~~	~~10^7 899-6~~	20	15.5	6							
1713	AA105283	10^7 899-6	ϕ									
2054	AA111331	10^7 899-6	14	9.5	4							
2057	AA111332	10^7 899-6	15	11.5	8							
1709	AA104549	10^7 3616	ϕ									
1723	AA104549	10^7 3616	ϕ									
~~2069~~	~~AA108968~~	~~10^7 3616~~										
~~2070~~	~~AA108968~~	~~10^7 3616~~										
2064	AA111334	10^7 3616	15.5	13.5	10.5							
2041	AA111334	10^7 3616	14	12	9							
1859 ϕ	AA108972	RT Alone	11	9.5	7							
1861 R	AA108972	RT Alone face	[illegible]	[illegible]	[illegible]	14.5	13	12.5				
1862 LR	AA108972	RT Alone	5.5	5	3.5							
1892 (L)	AA108964	RT Alone necrotic tail	6.5	6	3.5							
1863 ϕ	AA108971	10^7 3616+RT	6	5	4							
1864 L	AA108971	10^7 3616+RT	11	10	9.5							
1865 R	AA108971	10^7 3616+RT	ϕ									
1866 LR	AA108971	10^7 3616+RT	ϕ									
1883 ϕ	AA108966	10^7 3616+RT	ϕ									
1884 L	AA108966	10^7 3616+RT	2	2	2							
1886 LR	AA108966	10^7 3616+RT	ϕ									
2074 R	AA108966	10^7 3616+RT	8.5	10	5							
2032 ϕ	AA111326	10^7 3616+RT	4.5	5	4.5							
2033 L	AA111326	10^7 3616+RT	5.5	5	3							
2035 LR	AA111326	10^7 3616+RT	8	7	5							
2072 R	AA111326	10^7 3616+RT	ϕ									
2036 ϕ	AA111327	10^7 3616+RT	8.5	8	5							
2037 L	AA111327	10^7 3616+RT	4.5	4	2.5							
2039 LR	AA111327	10^7 3616+RT	8	7	6							
2073 R	AA111327	10^7 3616+RT	6.5	7	3							
1871 ϕ	AA108969	10^7 899-6+RT	ϕ									
1872 L	AA108969	10^7 899-6+RT	2.5	2.5	2							
1873 R	AA108969	10^7 899-6+RT	7	7.5	6							
1874 LR	AA108969	10^7 899-6+RT	ϕ									
~~1879~~	~~AA108967~~	~~10^7 899-6+RT~~										
1880 L	AA108967	10^7 899-6+RT	ϕ									
1881 R	AA108967	10^7 899-6+RT	ϕ									
1882 LR	AA108967	10^7 899-6+RT	ϕ									
2044 ϕ	AA111329	10^7 899-6+RT	4.5	4	2.5							
2045 L	AA111329	10^7 899-6+RT	ϕ									
2046 R	AA111329	10^7 899-6+RT	6.5	6	3							
2047 LR	AA111329	10^7 899-6+RT	ϕ									
2048 ϕ	AA111330	10^7 899-6+RT	3	3	2							
2049 L	AA111330	10^7 899-6+RT	[illegible]	[illegible]	[illegible]	13	12	10				
2050 R	AA111330	10^7 899-6+RT	ϕ									
2051 LR	AA111330	10^7 899-6+RT	10	9	6							

4/24/95

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0		23.3					
~~2075~~	~~AA105283~~	~~10^7 899-6~~	~~21~~	~~17~~	~~8~~		~~22.4~~					
1713	AA105283	10^7 899-6	0	0	0		21.4					
2054	AA111331	10^7 899-6	13.5	6.5	5		20.5					
2057	AA111332	10^7 899-6	18.5	16.5	14.5		23.3					
1709	AA104549	10^7 3616	0	0	0		24.0					
1723	AA104549	10^7 3616	0	0	0		23.5					
2041	AA111334	10^7 3616	18	16	14		24.8					
2064	AA111334	10^7 3616	18.5	16.5	12		23.5					
1859	AA108972	RT Alone	12	9.5	8.5		26.1					
~~1861~~	AA108972	RT Alone sac										
1862	AA108972	RT Alone	5.5	4.5	3.5		25.1					
1892	AA108964	RT Alone	5.5	5.5	3.5		24.1					
1863	AA108971	10^7 3616+RT	5	4.5	3.5		25.0					
1864	AA108971	10^7 3616+RT	11	14	11		25.1					
1865	AA108971	10^7 3616+RT	0	0	0		25.9					
1866	AA108971	10^7 3616+RT	0	0	0		25.3					
1883	AA108966	10^7 3616+RT	0	0	0		22.2					
1884	AA108966	10^7 3616+RT	2	2	1		24.4					
1886	AA108966	10^7 3616+RT	0	0	0		21.3					
2074	AA108966	10^7 3616+RT	8	9	6		16.3					
2032	AA111326	10^7 3616+RT	4.5	5.5	3.5		22.0					
2033	AA111326	10^7 3616+RT	5.5	4.5	3.5		24.8					
2035	AA111326	10^7 3616+RT	8	6.5	5		21.7					
2072	AA111326	10^7 3616+RT	0	0	0		24.2					
2036	AA111327	10^7 3616+RT	8	7	4		23.9					
2037	AA111327	10^7 3616+RT	4	4.5	1.5		24.6					
2039	AA111327	10^7 3616+RT	9	9	8		20.3					
2073	AA111327	10^7 3616+RT	7	7	4		20.2					
1871	AA108969	10^7 899-6+RT	5	5	3		23.3					
~~1872~~	AA108969	10^7 899-6+RT	ascites									
1873	AA108969	10^7 899-6+RT	6.5	8	5.5		23.8					
1874	AA108969	10^7 899-6+RT	0	0	0		26.1					
1880	AA108967	10^7 899-6+RT	0	0	0		22.4					
1881	AA108967	10^7 899-6+RT	0	0	0		25.4					
1882	AA108967	10^7 899-6+RT	0	0	0		24.4					
2044	AA111329	10^7 899-6+RT	3.5	3.5	3		23.3					
2045	AA111329	10^7 899-6+RT	0	0	0		25.9					
2046	AA111329	10^7 899-6+RT	5.5	6	2.5		24.8					
ace ~~2047~~	AA111329	10^7 899-6+RT	0	0	0		22.1					
2048	AA111330	10^7 899-6+RT	2.5	2.0	1		17.4					
2049	AA111330	10^7 899-6+RT	13.5	11	9		24.7					
2050	AA111330	10^7 899-6+RT	4	3	2		23.2					
2051	AA111330	10^7 899-6+RT	8.5	8.0	6.5		22.9					

4/27/95

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0							
~~2075~~	~~AA105283~~	~~10^7 899-6~~	16	12	3							
1713	AA105283	10^7 899-6	0	0	0							
2054	AA111331	10^7 899-6	11	5	3.5							
~~2057~~	AA111332	10^7 899-6	20	17.5	15							
1709	AA104549	10^7 3616	0	0	0							
~~1723~~	AA104549	10^7 3616	0	0	0							
~~2041~~	AA111334	10^7 3616	19	17	15							
~~2064~~	AA111334	10^7 3616	17.5	17	13.5							
1859	AA108972	RT Alone	12	11	8							
~~1861~~	AA108972	RT Alone										
1862	AA108972	RT Alone	5	4	3.5							
1892	AA108964	RT Alone	6	5	4							
1863	AA108971	10^7 3616+RT	5	5	3.5							
1864	AA108971	10^7 3616+RT	13	9.5	5.5							
1865	AA108971	10^7 3616+RT	0	0	0							
1866	AA108971	10^7 3616+RT	0	0	0							
1883	AA108966	10^7 3616+RT	0	0	0							
1884	AA108966	10^7 3616+RT	2	2.5	1							
1886	AA108966	10^7 3616+RT	0	0	0							
2074	AA108966	10^7 3616+RT	9.5	8.5	5							
2032	AA111326	10^7 3616+RT	4.5	4	3.5							
2033	AA111326	10^7 3616+RT	5.5	5	3							
2035	AA111326	10^7 3616+RT	8	6.5	4.5							
2072	AA111326	10^7 3616+RT	0	0	0							
2036	AA111327	10^7 3616+RT	8	7	4							
2037	AA111327	10^7 3616+RT	4	4	2							
2039	AA111327	10^7 3616+RT	9.5	8	6							
2073	AA111327	10^7 3616+RT	7	7	3							
1871	AA108969	10^7 899-6+RT	5.5	5	3							
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT	7.5	6	5							
1874	AA108969	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0							
1881	AA108967	10^7 899-6+RT	0	0	0							
1882	AA108967	10^7 899-6+RT	0	0	0							
2044	AA111329	10^7 899-6+RT	4	3.5	3							
2045	AA111329	10^7 899-6+RT	0	0	0							
2046	AA111329	10^7 899-6+RT	5	5	2.5							
2047	AA111329	10^7 899-6+RT										
2048	AA111330	10^7 899-6+RT	2	3	1.5							
2049	AA111330	10^7 899-6+RT	16	13	10							
2050	AA111330	10^7 899-6+RT	2	3	2							
2051	AA111330	10^7 899-6+RT	9	8	5.5							

518
17
12.6

306
13
18

5/1/95

Mouse #	Cage #	Group	Day 0				weight	Day 3				weight
1710	AA105283	10^7 899-6	0	0	0		24.0					
~~2075~~	~~AA105283~~	~~10^7 899-6~~	~~0~~	~~0~~	~~0~~		~~21.8~~					
1713	AA105283	10^7 899-6	3	2	1		21.8					
2054	AA111331	10^7 899-6	0	0	0		21.8					
2057	AA111332	10^7 899-6										
1709	AA104549	10^7 3616										
1723	AA104549	10^7 3616	0	0	0		23.7					
2041	AA111334	10^7 3616										
2064	AA111334	10^7 3616										
1859	AA108972	RT Alone	13	11.5	8		23.9					
1861	AA108972	RT Alone										
1862	AA108972	RT Alone	4.5	5	4		25.8					
1892	AA108964	RT Alone	5	5.5	4		24.3					
1863	AA108971	10^7 3616+RT	4.5	4	3		24.6					
1864	AA108971	10^7 3616+RT	13	10.5	7.5		26.3					
1865	AA108971	10^7 3616+RT	2.5	2.5	1		26.5					
1866	AA108971	10^7 3616+RT	3	3.5	1		25.8					
1883	AA108966	10^7 3616+RT	0	0	0		22.6					
1884	AA108966	10^7 3616+RT	1.5	1.5	1.5		24.3					
1886	AA108966	10^7 3616+RT	0	0	0		21.4					
2074	AA108966	10^7 3616+RT	9.5	8	6		17.3					
2032	AA111326	10^7 3616+RT	7	5	4		21.4					
2033	AA111326	10^7 3616+RT	4.5	4	3		24.3					
2035	AA111326	10^7 3616+RT	8	7	5.5		21.4					
2072	AA111326	10^7 3616+RT	0	0	0		23.5					
2036	AA111327	10^7 3616+RT	7	7	3		22.9					
2037	AA111327	10^7 3616+RT	3.5	3	2		24.7					
2039	AA111327	10^7 3616+RT	9	8	6.5		18.3					
2073	AA111327	10^7 3616+RT	6	5.5	2		19.5					
1871	AA108969	10^7 899-6+RT	4	4	3		23.7					
1872	AA108969	10^7 899-6+RT										
1873	AA108969	10^7 899-6+RT	6.5	6.5	3		23.6					
1874	AA108969	10^7 899-6+RT	0	0	0							
1880	AA108967	10^7 899-6+RT	0	0	0		21.8					
1881	AA108967	10^7 899-6+RT	0	0	0		26.6					
1882	AA108967	10^7 899-6+RT	0	0	0		23.5					
2044	AA111329	10^7 899-6+RT	4	3.5	2.5		22.9					
2045	AA111329	10^7 899-6+RT	0	0	0		25.9					
2046	AA111329	10^7 899-6+RT	4.5	4	3		23.8					
2047	AA111329	10^7 899-6+RT										
2048	AA111330	10^7 899-6+RT	3.5	3	1.5		16.8					
2049	AA111330	10^7 899-6+RT	15.5	13.5	11		26.4					
2050	AA111330	10^7 899-6+RT	2.5	2	1		23.2					
2051	AA111330	10^7 899-6+RT	9	7.5	5		22.6					

4/12/95

Apoptosis Assay

<u>Purpose</u> - To determine whether apoptosis plays a role in V-87 cell killing with TNF, RT, R3616 virus or R899-6 virus or combinations of these

<u>Methods</u> - 8 T150 flasks of U87 cells were grown to ~80% confluence.

		7am			1pm		5pm	1pm
		-6°	-4°	-2°	0°	2°	4°	→ 24°
	Control (no Rx)							Harvest
	TNF (100 ng/20 ml)				TNF			Harvest
	RT (20 Gy) + TNF				TNF		RT	Harvest
	RT (20 Gy)						RT	Harvest
3.8 µl	R3616 (10^7 PFU) + RT	infect					RT	Harvest
	R3616 (10^7 PFU)	infect						Harvest
2.6 µl	R899-6 (10^7 PFU) + RT	infect					RT	Harvest
	R899-6 (10^7 PFU)	infect						

1. Virally infected ~~cells~~ flasks were ~~a~~ treated with 10^7 PFU of virus (MOI approx 0.5) in 6 ml 199V media. Other flasks ~~received~~ incubated 6 ml of 199V $\bar{s}$ virus as control.

2. At 2 hours, an additional ~~24~~ 14 ml of ~~10~~% FCS media was added to bring total volume to ~~30~~ 20 cc
(actually I added 10% FCS by mistake x 3 hrs, switched to ØFCS @ 12N, this required decanting media from infected flasks)

3. Add TNF to appropriate flasks = .01 mg/ml stock = .01 µg/µl
add 10 µl (100 ng) to 20 ml

APOPTOSIS ASSAY

4/24/95

Sample	Apoptosis (%)
Baseline	2.3
Positive Control	80.4
No Treatment (-TdT)	0.3
No Treatment	1.9
RT 20 Gy	19.3
TNF	2.6
TNF + RT	19
R3616 (MOI=0.5)	29.3
R3616 + RT	23.2
R899-6 (MOI=0.5)	26.8
R899-6 + RT	11.4

U87

Neg. Control
No TdT

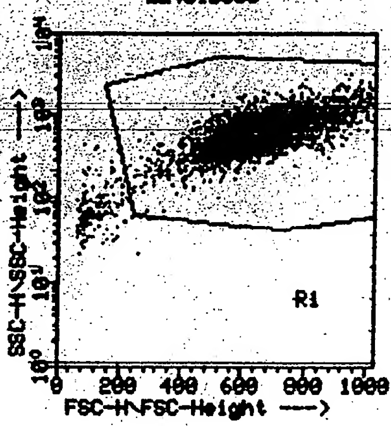
BECTON
DICKINSON

LYSYS II Ver 1.1 2/5/92

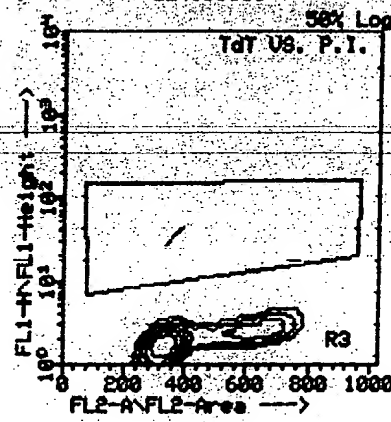
DATE: 24-APR-95 TIME: 14:54:28

SELECTED PREFERENCES: Arithmetic/Linear

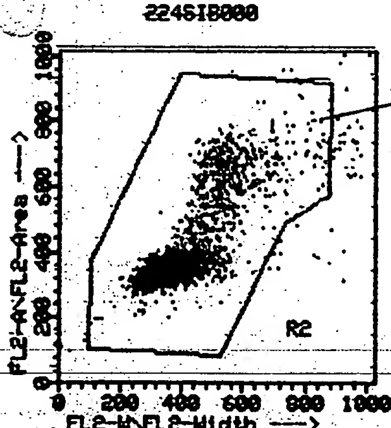
224SIB000



224SIB000



224SIB000



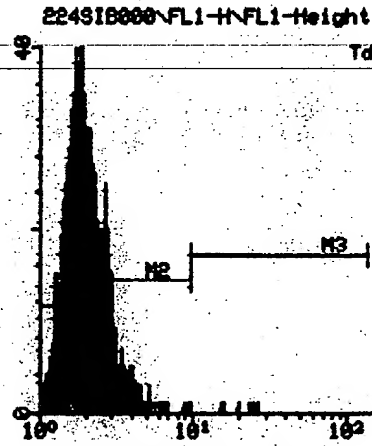
224SIB000

---- Region Stats ----

File: 224SIB000 Sample: GREG SIBLE
Date: 4/24/95 Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2368

Rgn	Events	% Gated	% Total
1 R1	2368	100.00	78.93
2 R2	2368	100.00	78.93
3 R3	8	0.34	0.27

224SIB000\FL1-H\FL1-Height



224SIB000\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB000

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2369	100.00	43	1.91
1	1.00, 2.81	2100	89.02	43	1.84
2	2.81, 9.82	273	11.53	20	3.16
3	9.82, 136	7	0.30	1	19.81

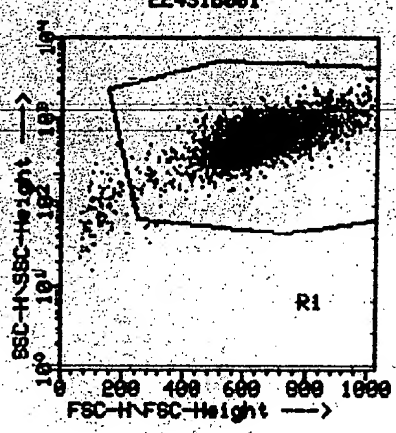
Control

BECTON DICKINSON LYSYS II Ver 1.1 2/6/92

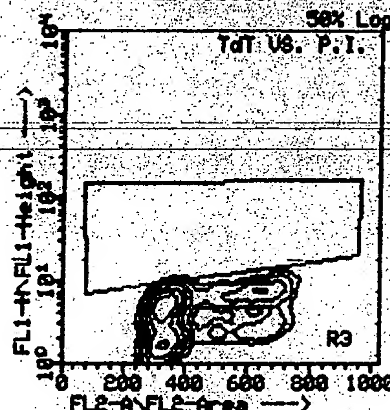
DATE: 24-APR-95 TIME: 14:56:24

SELECTED PREFERENCES: Arithmetic/Linear

224SIB001

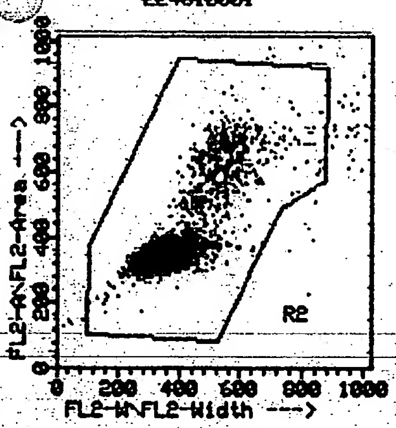


224SIB001



serum deplated

224SIB001



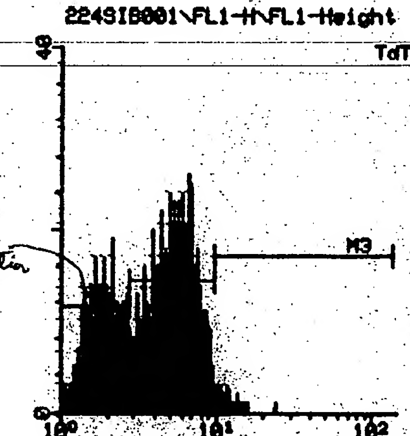
224SIB001

----- Region Stats -----

File: 224SIB001 Sample: GREG SIBLE'
Date: 4/24/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2514

Rgn	Events	% Gated	% Total
1 R1	2514	100.00	83.80
2 R2	2514	100.00	83.80
3 R3	13	0.52	0.43

224SIB001\FL1-H\FL1-Height



Chromatin condensation

224SIB001\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB001
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2514	100.00	26	4.33
1	1.00, 2.81	819	32.58	19	1.91
2	2.81, 9.82	1657	65.91	26	5.33
3	9.82, 136	48	1.91	3	11.09

RT

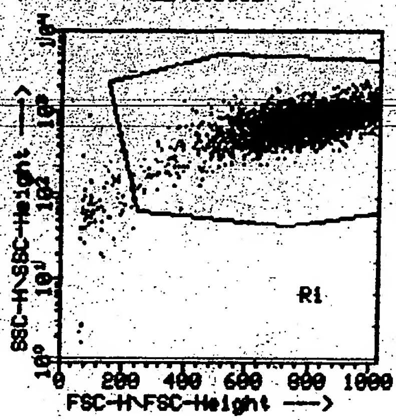
BECTON
DICKINSON

LYSYS II Ver 1.1 2/6/92

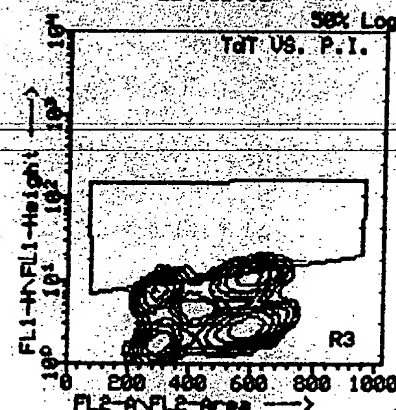
DATE: 24-APR-95 TIME: 14:58:36

SELECTED PREFERENCES: Arithmetic/Linear

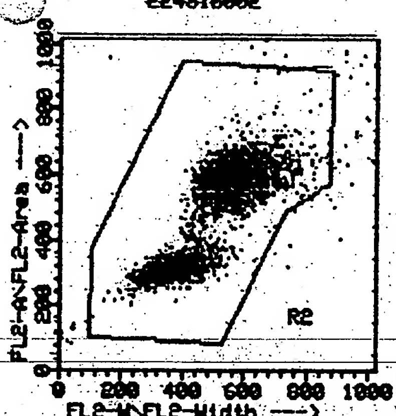
224SIB002



224SIB002



224SIB002



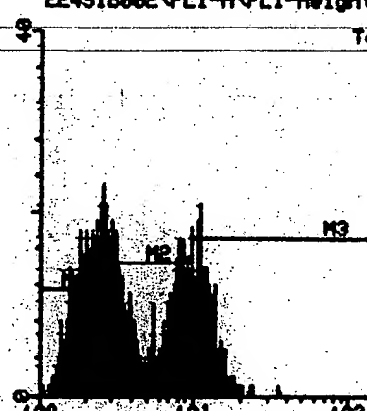
224SIB002

---- Region Stats ----

File: 224SIB002 Sample: GREG SIBLE'
Date: 4/24/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2343

Rgn	Events	% Gated	% Total
1 R1	2343	100.00	78.10
2 R2	2343	100.00	78.10
3 R3	209	8.92	6.97

224SIB002\FL1-H\FL1-Height



224SIB002\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB002

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

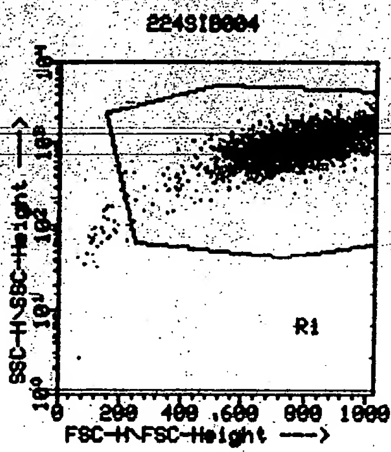
M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2343	100.00	23	3.34
1	1.00, 2.81	937	39.99	23	2.15
2	2.81, 9.82	974	41.57	19	5.03
3	9.82, 136	451	19.25	21	11.06

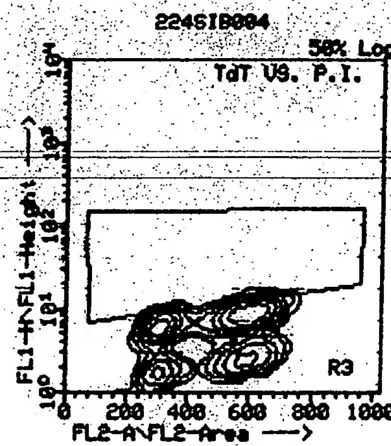
TNF + RT

BECTON DICKINSON LYSYS II Ver 1.1 2/5/92

DATE: 24-APR-95 TIME: 15:01:05

SELECTED PREFERENCES: Arithmetic/Linear



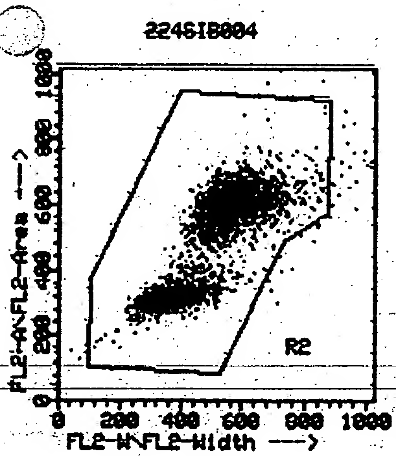


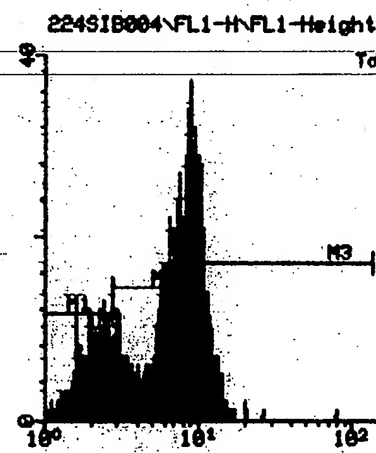
224SIB004

—— Region Stats ——

File: 224SIB004 Sample: GREG SIBLE
Date: 4/24/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2530

Rgn		Events	% Gated	% Total
1	R1	2530	100.00	84.33
2	R2	2530	100.00	84.33
3	R3	61	2.41	2.03





224SIB004\FL1-H\FL1-Height

— Arithmetic Histogram Statistics for 224SIB004

Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2530	100.00	37	7.17
1	1.00, 2.01	511	20.20	13	2.07
2	2.01, 9.02	1571	62.09	37	7.30
3	9.02, 136	401	19.01	29	10.75

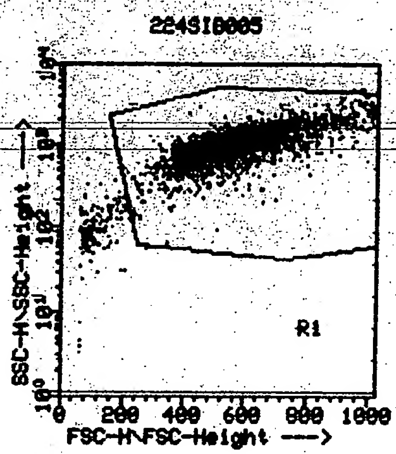
3616

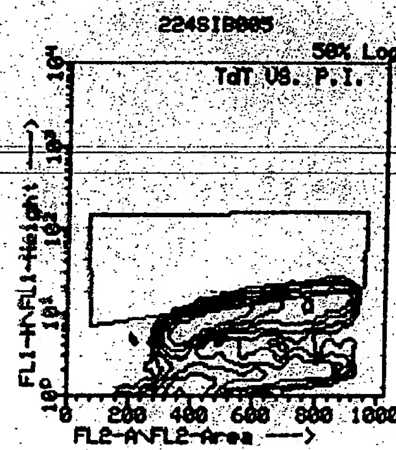
BECTON DICKINSON

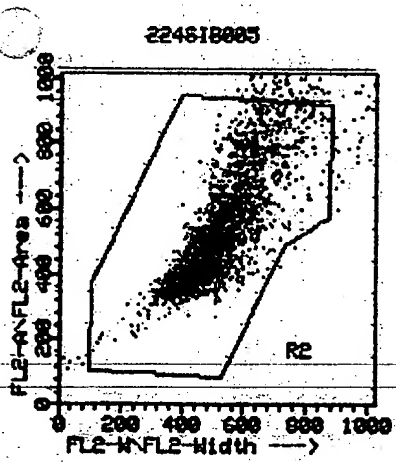
LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95 TIME: 15:01:58

SELECTED PREFERENCES: Arithmetic/Linear







224SIB005

----- Region Stats -----

File: 224SIB005 Sample: GREG SIBLE

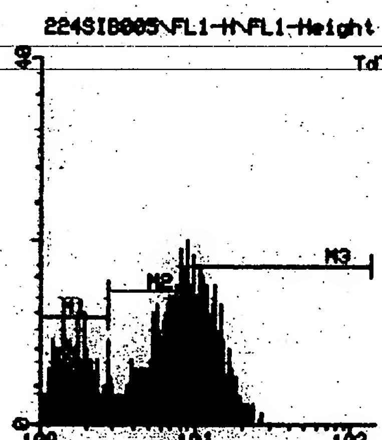
Date: 4/24/95 Gate G2= R1ANDR2

Selected Preference: Arithmetic/Lin

Parameters: FL2-A(LIN),FL1-H(LOG)

Total= 3000 Gated= 2002

Rgn	Events	% Gated	% Total
1 R1	2002	100.00	66.73
2 R2	2002	100.00	66.73
3 R3	189	9.44	6.30



224SIB005\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB005

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2002	100.00	20	7.17
1	1.00, 2.81	524	26.17	20	1.61
2	2.81, 9.82	909	45.40	20	6.98
3	9.82, 136	587	29.32	17	12.30

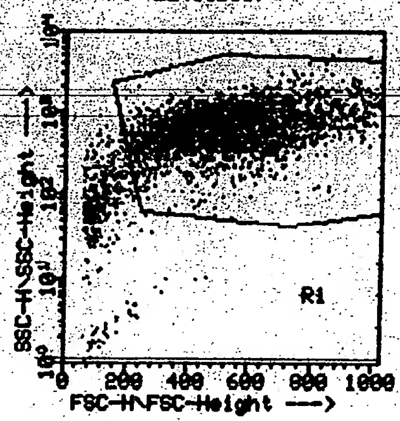
899-6

BECTON DICKINSON LYSYS II Ver 1.1 2/6/92

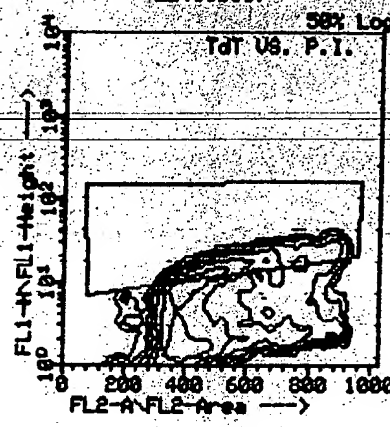
DATE: 24-APR-95 TIME: 15:03:40

SELECTED PREFERENCES: Arithmetic/Linear

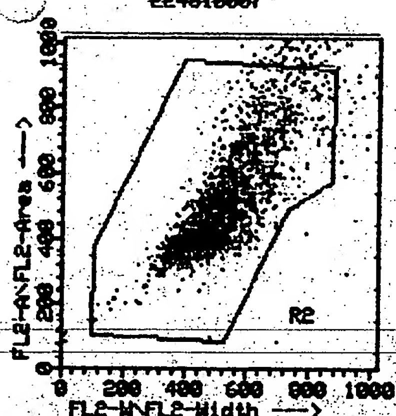
224SIB007



224SIB007



224SIB007



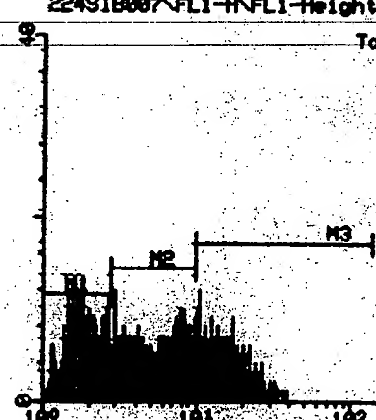
224SIB007

------ Region Stats ------

File: 224SIB007 Sample: GREG SIBLE'
Date: 4/24/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1710

Rgn	Events	% Gated	% Total
1 R1	1710	100.00	57.00
2 R2	1710	100.00	57.00
3 R3	366	21.40	12.20

224SIB007\FL1-H\FL1-Height



224SIB007\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB007
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1710	100.00	13	4.49
1	1.00, 2.81	599	35.03	13	1.64
2	2.81, 9.82	658	38.48	12	5.33
3	9.82, 136	459	26.84	12	14.20

899-6 + RT

BECTON DICKINSON

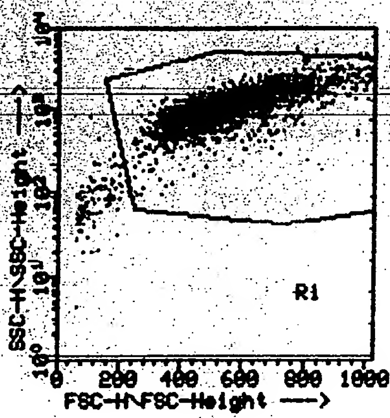
LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95

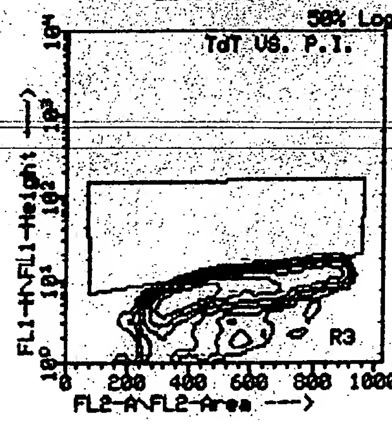
TIME: 15:04:34

SELECTED PREFERENCES: Arithmetic/Linear

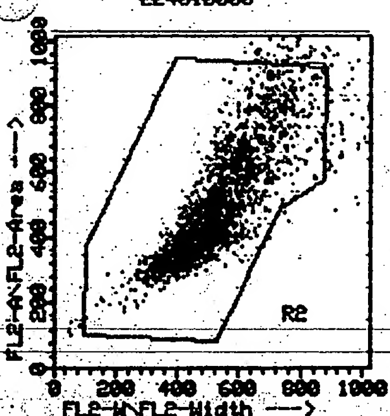
224SIB008



224SIB008



224SIB008



224SIB008

----- Region Stats -----

File: 224SIB008 Sample: GREG SIBLE

Date: 4/24/95 Gate G2= R1ANDR2

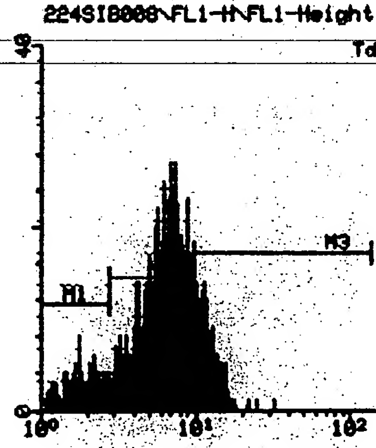
Selected Preference: Arithmetic/Lin

Parameters: FL2-A(LIN),FL1-H(LOG)

Total= 3000 Gated= 2068

Rgn	Events	% Gated	% Total
1 R1	2068	100.00	68.93
2 R2	2068	100.00	68.93
3 R3	30	1.45	1.00

224SIB008\FL1-H\FL1-Height



224SIB008\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224SIB008

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2068	100.00	27	6.73
1	1.00, 2.01	218	10.54	8	1.84
2	2.01, 9.82	1515	73.26	27	6.49
3	9.82, 136	349	16.88	14	11.44

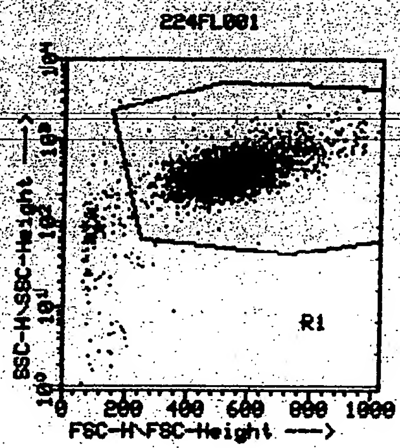
Baseline for Positive Control

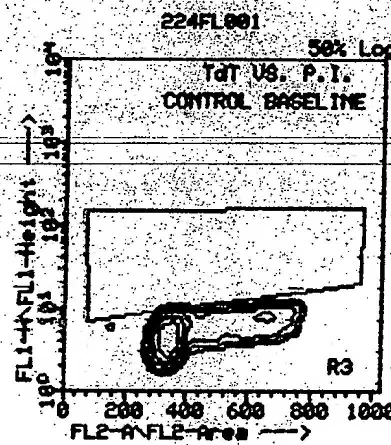
BECTON DICKINSON

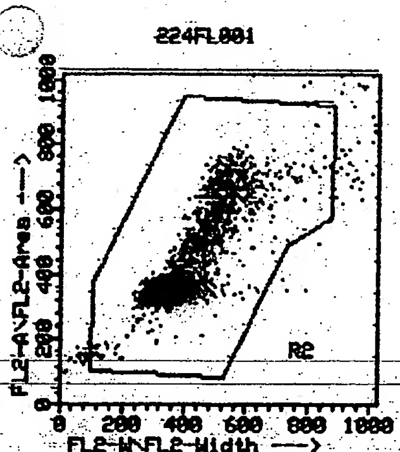
LYSYS II Ver 1.1 2/6/92

DATE: 24-APR-95 TIME: 15:11:28

SELECTED PREFERENCES: Arithmetic/Linear





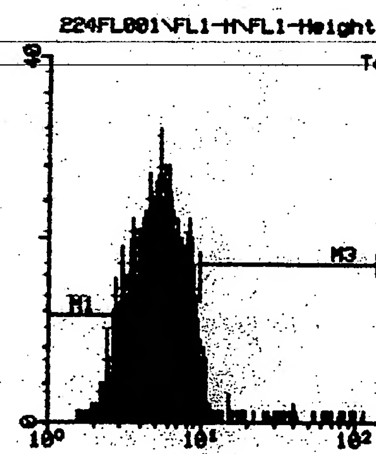


224FL001

---- Region Stats ----

File: 224FL001 Sample: FL5.12 CONT
Date: 4/24/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Lin
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2487

Rgn	Events	% Gated	% Total
1 R1	2487	100.00	82.90
2 R2	2487	100.00	82.90
3 R3	58	2.33	1.93



224FL001\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 224FL001
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2487	100.00	32	5.23
1	1.00, 2.01	151	6.07	12	2.57
2	2.01, 9.82	2253	90.59	32	5.28
3	9.82, 136	97	3.90	8	11.14

THE UNIVERSITY OF CHICAGO
ANIMAL RESOURCES CENTER

REQUEST FOR TRANSFER OF ANIMAL OWNERSHIP

Requested transfer date 4/18/95

We request the following animals be transferred:

SPECIES nude mice QUANTITY 56

CAGE CARD NUMBER(S)
AA113928 through AA113943 (16 cages)

REQUESTED TRANSFER FROM:

PRINCIPAL INVESTIGATOR

PROTOCOL FAS ACCOUNT

PHONE SIGNATURE

this animal(s) been used in research or teaching? YES NO

If yes how ?
The number of unused animals transferred will be restored to the number available for ordering under this ACUP.

REQUESTED TRANSFER TO:

PRINCIPAL INVESTIGATOR HALLAHAN / SIBLEY

PROTOCOL FAS ACCOUNT

PHONE 2-0294 SIGNATURE

Will this animal(s) be moved to a new housing site / room? YES NO

If yes where? CARLSON J-013
The number of animals transferred will be deducted from the number available for ordering under this ACUP.

ARC APPROVALS:

CLINICAL VETERINARIAN APPROVAL DATE
Animals which have been used or which are to be moved to a different housing site require the approval of a clinical veterinarian.

BUSINESS OFFICE APPROVAL DATE

ANSACTION DATE GCS ENTRY BY

(2/95)

For committee use:

ACUP No.: 58671B

ACUP Amendment–Supplemental Form A

Only the primary investigator of an ACUP is permitted to make changes to that ACUP. Please note that certain changes in an ACUP may affect other aspects of that ACUP, and should be reflected in this amendment. ACUPs with amendments require Institutional Animal Care and Use Committee (IACUC) review and approval. The IACUC reserves the right to determine whether proposed changes are substantive or not, and to request further information or a new ACUP application, as appropriate. When submitting an amendment, the Principal Investigator is required to review all of the details of the original ACUP and to assure the IACUC that all unamended details remain identical to the original ACUP. Please note that the amendment must be typed.

Investigator: Dennis Hallahan, M.D. **Department:** Radiation Oncology

ACUP Number: 58671B **Original approval date:**

The following changes are herein proposed for this protocol:

[ ] staff involved

Note: Include the number of years of work experience with the species used in this protocol, and confirm that each individual has reviewed the **University of Chicago Manual on Laboratory Animals Interim Guide.** If anyone listed has less than one year of relevant experience, please briefly describe how they will be or have been trained.

[ ] housing [X] procedure [ ] agents used

[ ] number of animals [ ] change in co-investigator

[ ] site outside centrally managed animal facilities to which live animals are taken and route of animal transport

[ ] response to questions raised during the IACUC-ARC review process [ ] other

Provide sufficient details, including a rationale for the proposed change, in narrative form to allow the committee to make a decision. Use additional pages as necessary and <u>submit revised pages of the original ACUP, as appropriate.</u>

Clarification of endpoints used in protocol #58671B:

<u>1.) Tumor Size:</u> The cutoff as mentioned in the protocol is "10% of body weight". Tumor measurements are typically performed twice weekly and tumor volumes are estimated by the formula **1/2(length x width x height)**. This is is derived from the equation for the volume of a sphere= $4\pi r^3/3 = 4r^3 = d^3/2 = (l \times w \times h)/2$. Since most nude mice weigh 18-25 grams, a tumor size of **2000 mm^3** = 2 cc = 2 grams is approximately 10% of body weight. This will be used as the cutoff for tumor size and can be easily verified by those monitoring the animals.

<u>2.) Ulceration of tumors:</u> When rapidly growing tumors outgrow their blood supply, they typically undergo necrosis in the central portion of the tumor. This creates a necrotic cavity which may heal completely over time if the tumor is cured. This situation is also seen in humans, most commonly in patients undergoing treatment for head and neck cancers. In our experience, this central necrosis is not tender in nude mice and has not predisposed the animals to infection, weight loss or loss of limb function. We continue to use these latter three conditions as criteria to sacrifice the animals.

Dennis Hallahan 4/18/95

Investigator Date

This form must be submitted bearing the original signature of the investigator.

01 ACC–ARC 2/7/95

The University of Chicago Departmental Purchase Order

Purchase Order Number
Z890627
THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

VWR Scientific
P.O. Box 66929
O'Hare AMF
Chicago — City | IL — State | 60666 — Zip Code

Payment Terms ____ Delivery charge? ☐ Yes ☒ No

Telephone No. 1-800-932-5000 FAX No. ____

Ship To

THE UNIVERSITY OF CHICAGO | Dept. Code: ____

Radiation Oncology — Department
Greg Sibley, MD — Attn: | 6-03 — Room
5830 S. Ellis Ave
Chicago — City | IL — State | 60637 — Zip Code

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ____ Payroll No. ____

Print Name ____ Ext. ____

Account Code ____ Date ____

Order placed by phone? ☐ No ☒ Yes

Sharon — Order placed with (name) | 4/24/95 — Date

Greg Sibley — Order placed by (name) | 2-0294 — Phone #

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
4	1	Vernier Calipers 19155-057	49.88	199.52
		VWR Acct #77055-172		

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

ORDER TOTAL ____

Form ZDPO 100 07/94

THE UNIVERSITY OF CHICAGO **ANIMAL RESOURCES CENTER** **ANIMAL PROCUREMENT REQUEST** (#315B)

REQ NO 24129

ARC USE ONLY

P.O. # ____

ORDER DATE ____

REF. # ____

CONTACT ____

EST AMT ____

SCHED DEL ____

SPECIAL ROUTING

NON COM VENDOR ____

FLAGGED BY PROTOCOL ____

PROCUREMENT DESK: 2-9364

TO BE COMPLETED BY REQUESTING PARTY

☐ STANDING ORDER: ____ SHIPMENTS ON A ____ BASIS

REQUEST BY: Greg Sibley, MD DATE: 4/26/95

REQUESTORS PHONE NUMBER: 2-0294

AUTHORIZED SIGNATURE: [signature]

FAS ACCOUNT: 2-73731-5100 P.I.: Hallam

VENDOR: FCRI PROTOCOL: 5861

REQUESTED DELIVERY DATE: 5/3/95 PHONE: 2-0294

SPECIES: Mouse QUANTITY: 80

STRAIN: Athymic Nude SEX: M F EITHER

WEIGHT/AGE: 5-6 wks ALTERNATE WEIGHT/AGE: ____
(IF NO ALTERNATE IS INDICATED AND FIRST CHOICE IS NOT AVAILABLE THIS REQUEST WILL BE RETURNED TO REQUESTOR)

MICROISOLATOR TOP: YES ☒ NO ☐ (RODENTS ONLY)

SPECIAL REQUIREMENTS: Mice to go to Carlson Biohazard Suite Room J-013

HOUSE AT: X CARLSON J-013 ____ WYLER ~~X~~ CLSC ____ FMI ____ OTHER

5/1/95

Apoptosis Assay - U87 Cells

Purpose - To see if apoptosis is seen in virally treated groups at earlier timepoints and to see if apoptosis is seen with TNF+RT at other time points

Methods - T-150 flasks of U87 cells were grown to ~80% confluence

			7^{AM} -10	1^{PM} -4	5^{pm} 0°	11^{PM} 6°	5^{pm} 24°	5^{pm} 48°	5^{pm} 72°	%A
	Control						Harvest			1.8
2	TNF+RT	6°		TNF	RT	Harvest				2.6
3	"	24°		TNF	RT		Harvest			10..
4		48°		TNF	RT			Harvest		5
5		72°		TNF	RT				Harvest	16.
6	10^7 899-6	6°	infect			Harvest				4
10		24°	infect				Harvest			13.
7	10^7 899-6+RT	6°	infect		RT	Harvest				5.6
11		24°	infect		RT		Harvest			25
8	10^8 899-6	6°	infect			Harvest				13.7
12		24°	infect				Harvest			11.
9	10^8 899-6+RT	6°	infect		RT	Harvest				8..
13		24°	infect		RT		Harvest			9

Infection - cells were infected in 6 ml of 199V media x 2°
then additional 14 cc of no FCS media was added

RT - Single fraction of 20 Gy

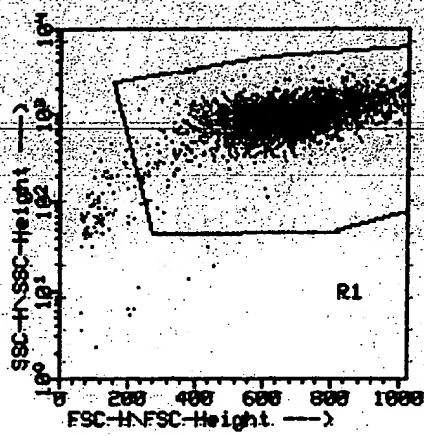
TNF - 10 ul of 0.01 mg/ml stock in 20 ml media

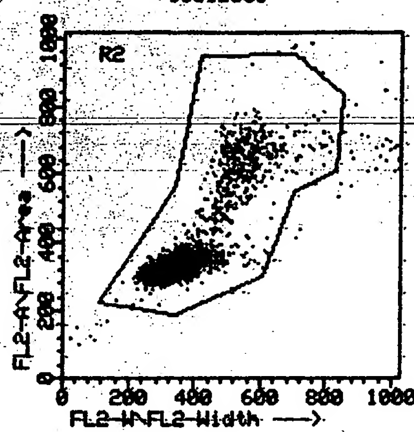
Control + TdT

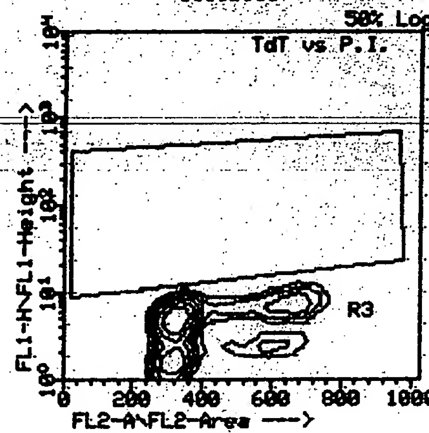
BECTON DICKINSON LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95 TIME: 15:04:24

SELECTED PREFERENCES: Arithmetic/Linear







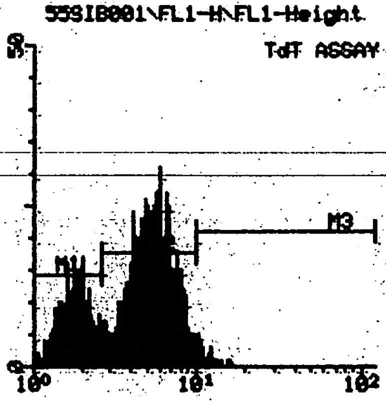
55SIB001

----- Region Stats -----

File: 55SIB001 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2399

Rgn	Events	% Gated	% Total
1 R1	2399	100.00	79.97
2 R2	2399	100.00	79.97
3 R3	1	0.04	0.03

55SIB001\FL1-H\FL1-Height

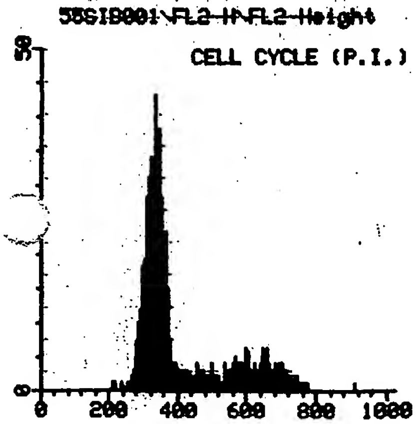


55SIB001\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB001
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2399	100.00	31	4.74
1	1.00, 2.64	646	26.93	17	1.78
2	2.64, 9.91	1717	71.57	31	5.42
3	9.91, 118	44	1.83	5	10.89

55SIB001\FL2-H\FL2-Height



1.8

TNF+RT 6°

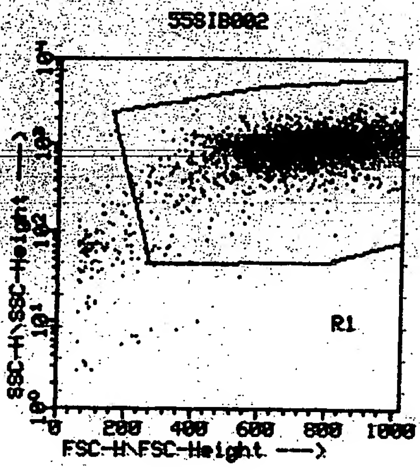
BECTON
DICKINSON

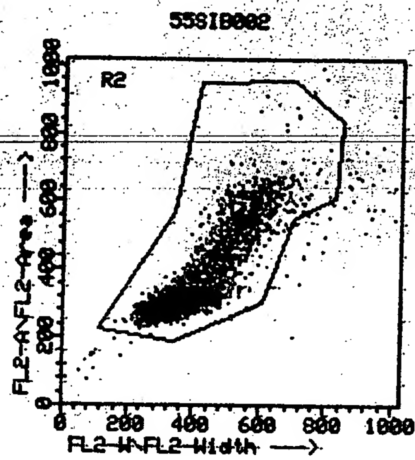
LYSYS II Ver 1.1 2/6/92

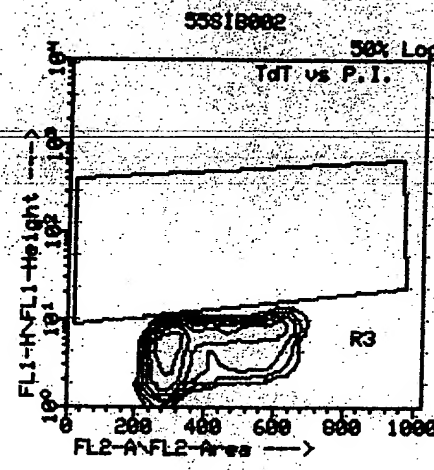
DATE: 5-MAY-95

TIME: 15:05:22

SELECTED PREFERENCES: Arithmetic/Linear





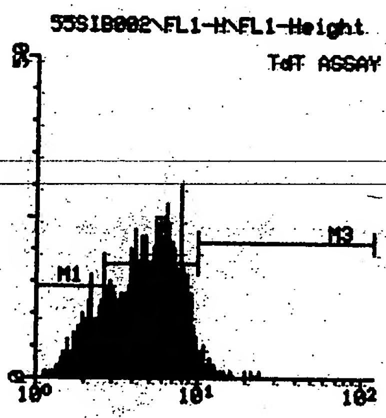


55SIB002

—— Region Stats ——

File: 55SIB002 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preferences: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2450

Rgn	Events	% Gated	% Total	:
1 R1	2450	100.00	81.67	1:
2 R2	2450	100.00	81.67	1:
3 R3	4	0.16	0.13	1:



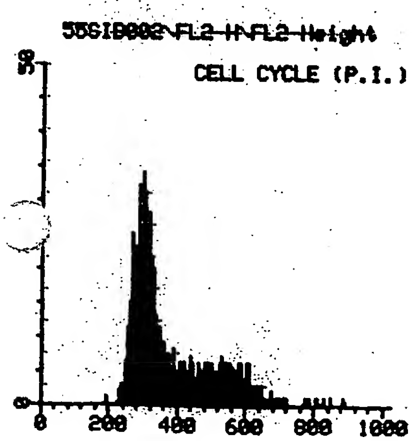
55SIB002\FL1-H\FL1-Height

—— Arithmetic Histogram Statistics for 55SIB002

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2450	100.00	30	4.83
1	1.00, 2.64	400	16.33	16	2.15
2	2.64, 9.91	2003	81.76	30	5.28
3	9.91, 110	63	2.57	5	10.84



2.6

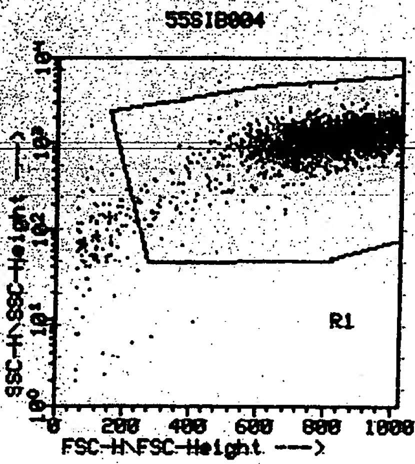
TNFtRT 480

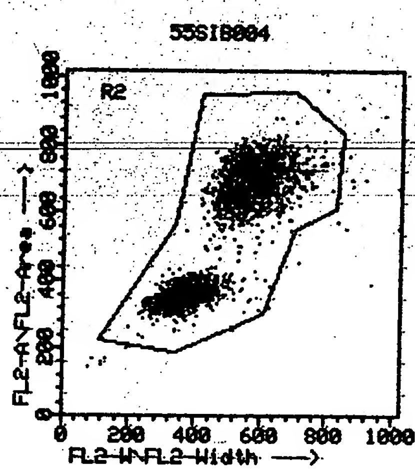
BECTON
DICKINSON

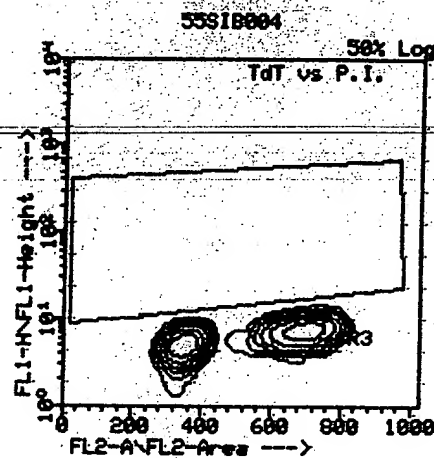
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95 TIME: 15:07:20

SELECTED PREFERENCES: Arithmetic/Linear





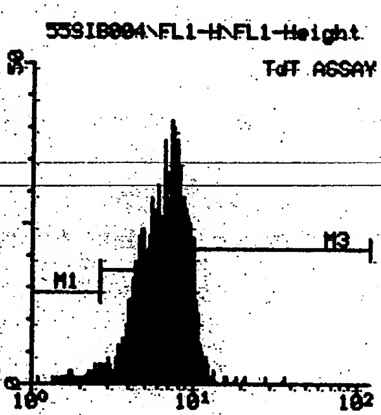


55SIB004

----- Region Stats -----

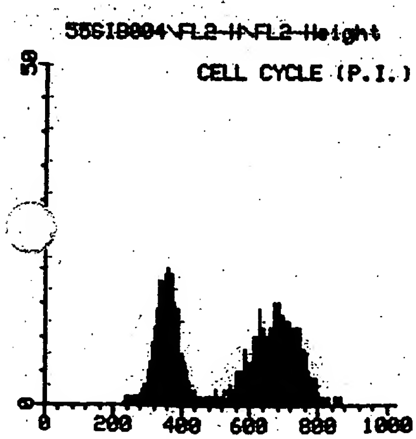
File: 55SIB004 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2479

Rgn	Events	% Gated	% Total	:
1 R1	2479	100.00	82.63	2
2 R2	2479	100.00	82.63	1;
3 R3	4	0.16	0.13	1·



55SIB004\FL1-H\FL1-Height
--- Arithmetic Histogram Statistics for 55SIB004
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2479	1[illegible].00	41	6.61
1	1.00, 2.64	35	41	3	2.09
2	2.64, 9.91	23[illegible]	[illegible].03	41	6.55
3	9.91, 118	[illegible]	[illegible].04	13	10.46



5

TNF+RT 72°

BECTON DICKINSON

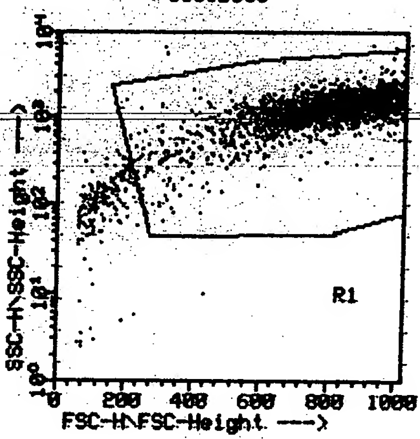
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

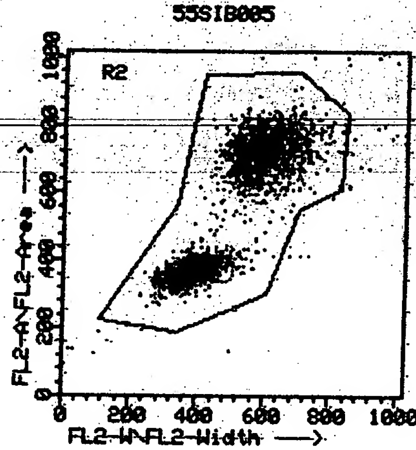
TIME: 15:08:20

SELECTED PREFERENCES: Arithmetic/Linear

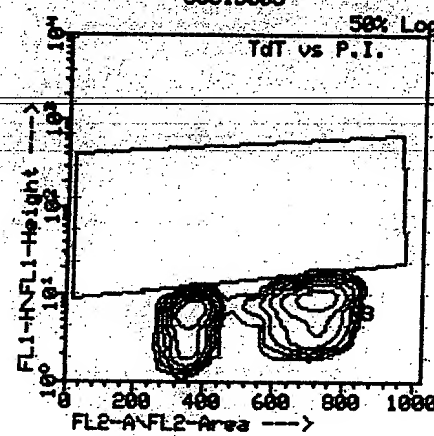
55SIB005



55SIB005



55SIB005



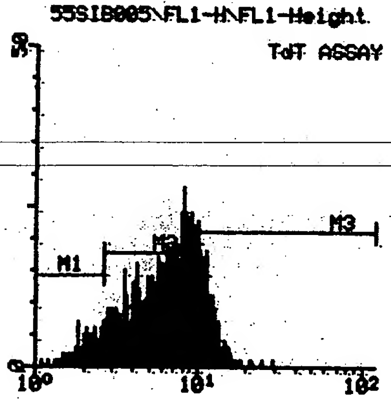
55SIB005

----- Region Stats -----

File: 55SIB005 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 2162

Rgn	Events	% Gated	% Total	:
1 R1	2162	100.00	72.07	[illegible]
2 R2	2162	100.00	72.07	[illegible]
3 R3	7	0.32	0.23	[illegible]

55SIB005\FL1-H\FL1-Height

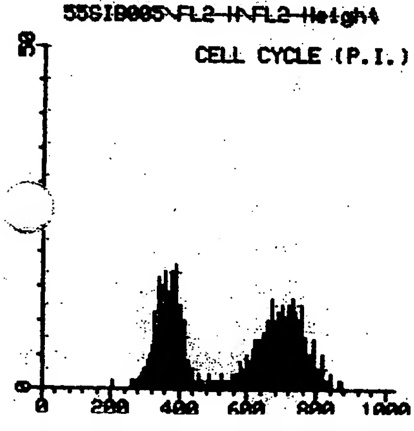


55SIB005\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB005
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2162	100.00	28	6.73
1	1.00, 2.64	167	7.72	7	2.19
2	2.64, 9.91	1656	76.60	28	6.38
3	9.91, 118	360	16.65	18	11.14

55SIB005\FL2-H\FL2-Height



16.7

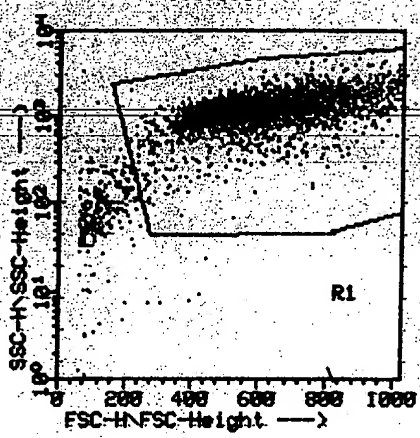
2x10 7 889-6- 6°

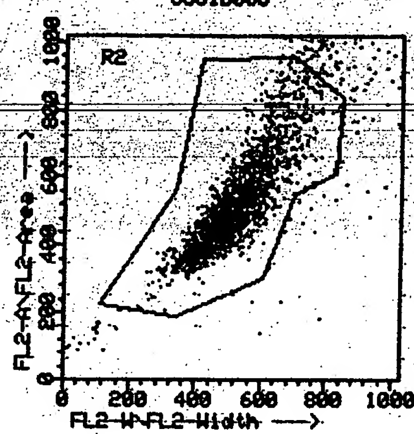
BECTON
DICKINSON

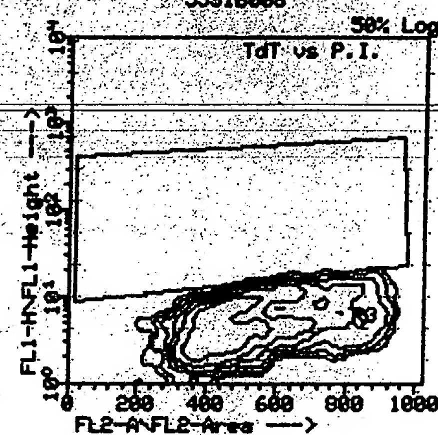
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95 TIME: 15:11:13

SELECTED PREFERENCES: Arithmetic/Linear





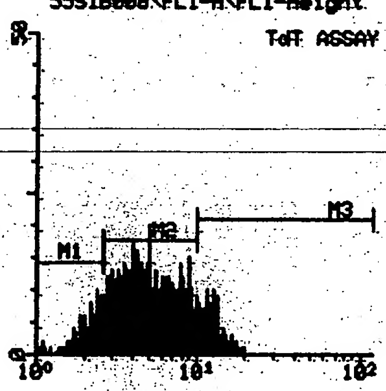


55SIB008

—— Region Stats ——

File: 55SIB008 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1748

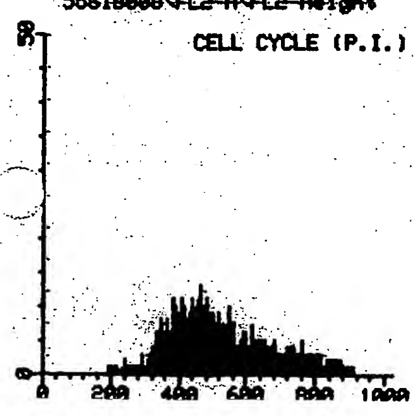
Rgn	Events	% Gated	% Total	:
1 R1	1748	100.00	58.27	1
2 R2	1748	100.00	58.27	1
3 R3	2	0.11	0.07	1



55SIB008\FL1-H\FL1-Height

—— Arithmetic Histogram Statistics for 55SIB008
Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1748	100.00	20	4.78
1	1.00, 2.64	225	12.87	10	2.23
2	2.64, 9.91	1298	74.26	20	4.74
3	9.91, 110	240	13.73	10	11.92



13,7

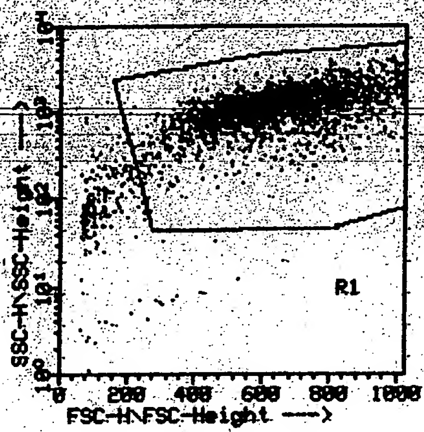
2xW 859-6+RT 6°

BECTON DICKINSON LYSYS II Ver 1.1 2/6/92

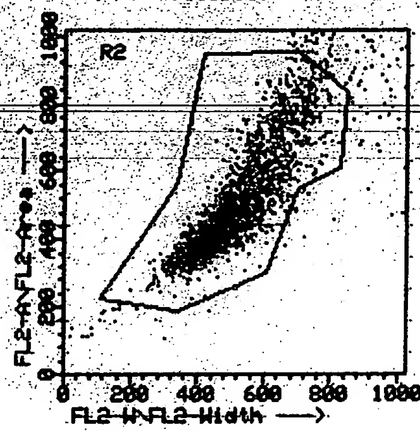
DATE: 5-MAY-95 TIME: 15:12:14

SELECTED PREFERENCES: Arithmetic/Linear

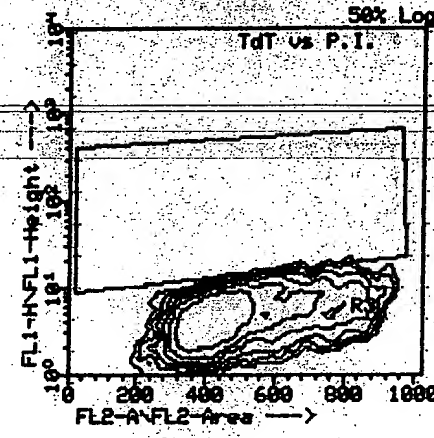
55SIB009



55SIB009



55SIB009



55SIB009

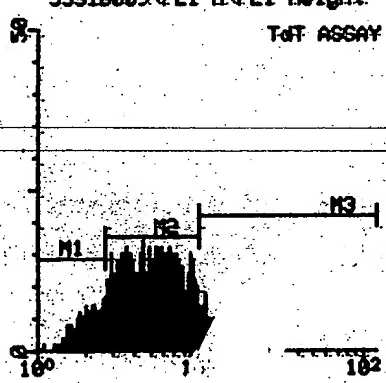
—— Region Stats ——

File: 55SIB009 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1817

Rgn	Events	% Gated	% Total	
1 R1	1817	100.00	60.57	1
2 R2	1817	100.00	60.57	1
3 R3	6	0.33	0.20	1

55SIB009\FL1-H\FL1-Height

TdT ASSAY



55SIB009\FL1-H\FL1-Height

—— Arithmetic Histogram Statistics for 55SIB009

Selected Preferences: Arithmetic/Linear

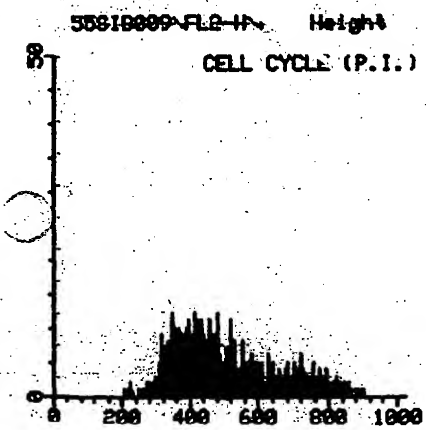
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1817	100.00	17	5.00
1	1.00, 2.64	236	12.99	8	2.13
2	2.64, 9.91	1435	78.98	17	5.19
3	9.91, 118	156	8.59	9	11.44

55SIB009\FL2-H\ Height

CELL CYCLE (P.I.)

8.6



2x10 6 888-6 + RT 24°

BECTON
DICKINSON

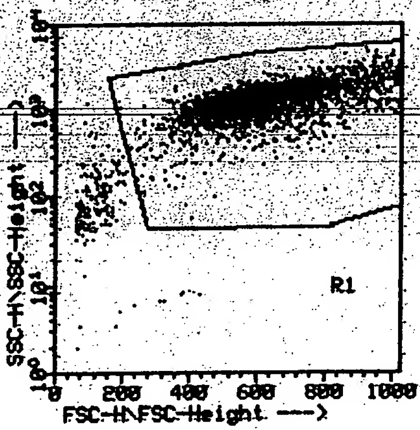
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

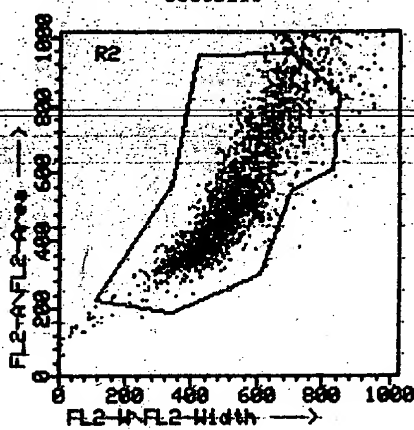
TIME: 15:14:11

SELECTED PREFERENCES: Arithmetic/Linear

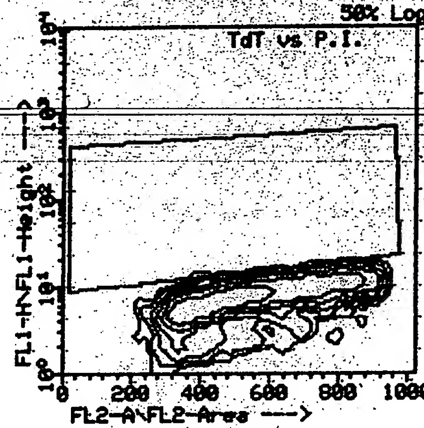
55SIB011



55SIB011



55SIB011



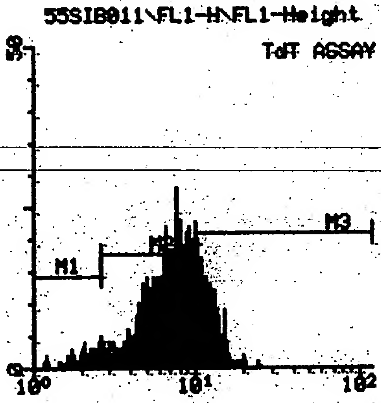
55SIB011

----- Region Stats -----

File: 55SIB011 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1815

Rgn	Events	% Gated	% Total	:
1 R1	1815	100.00	60.50	1
2 R2	1815	100.00	60.50	1
3 R3	5	0.28	0.17	1

55SIB011\FL1-H\FL1-Height



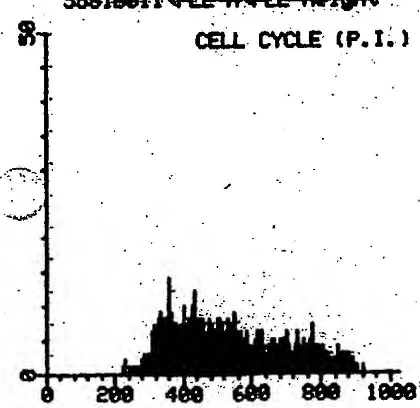
55SIB011\FL1-H\FL1-Height

---- Arithmetic Histogram Statistics for 55SIB011

Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1815	100.00	28	7.64
1	1.00, 2.64	95	5.23	5	2.09
2	2.64, 9.91	1290	71.07	28	6.92
3	9.91, 118	454	25.01	28	11.55

55SIB011\FL2-H\FL2-Height



25

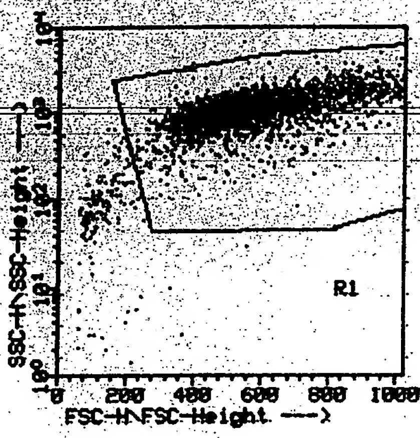
2x10⁷ 888-6   24°

BECTON DICKINSON   LYSYS II Ver 1.1 2/6/92

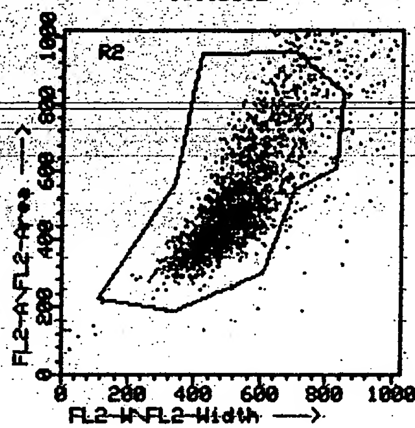
DATE: 5-MAY-95   TIME: 15:15:11

SELECTED PREFERENCES: Arithmetic/Linear

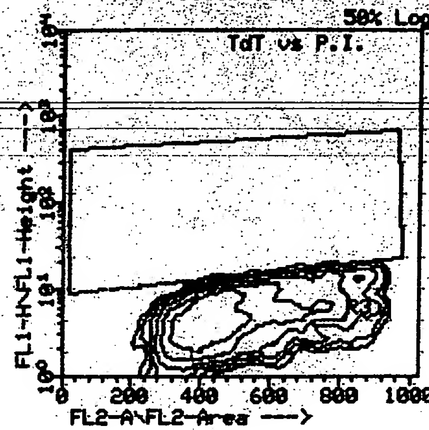
55SIB012



55SIB012



55SIB012



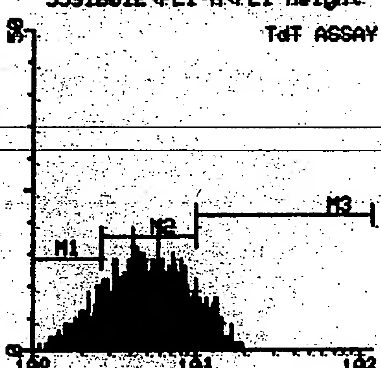
55SIB012

----- Region Stats -----

File: 55SIB012 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1795

Rgn	Events	% Gated	% Total	:
1 R1	1795	100.00	59.83	1:
2 R2	1795	100.00	59.83	1:
3 R3	3	0.17	0.10	1

55SIB012\FL1-H\FL1-Height



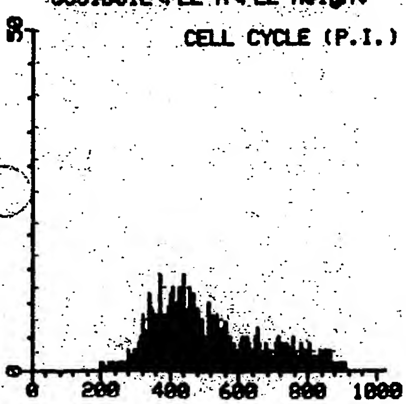
55SIB012\FL1-H\FL1-Height

--- Arithmetic Histogram Statistics for 55SIB012

Selected Preferences: Arithmetic/Linear
Parameter FL1-H FL1-Height   Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1795	100.00	19	4.91
1	1.00, 2.64	269	14.99	10	2.11
2	2.64, 9.91	1339	74.60	19	5.09
3	9.91, 118	205	11.42	11	11.65

55SIB012\FL2-H\FL2-Height



11.4

2×10^7 8846 + RT 24

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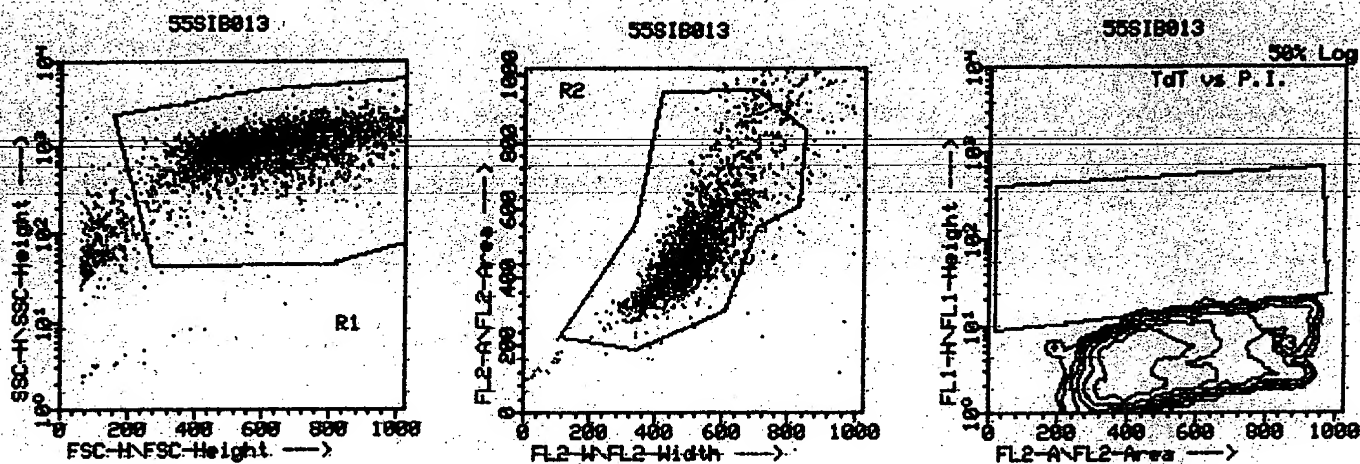
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

TIME: 15:16:10

SELECTED PREFERENCES: Arithmetic/Linear

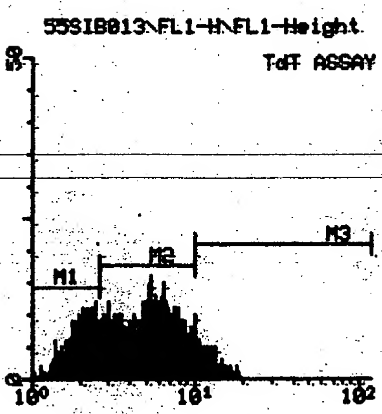


55SIB013

----- Region Stats -----

File: 55SIB013 Sample: SIBLEY APOP !
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1622

Rgn	Events	% Gated	% Total	:
1 R1	1622	100.00	54.07	1
2 R2	1622	100.00	54.07	1
3 R3	4	0.25	0.13	1



55SIB013\FL1-H\FL1-Height

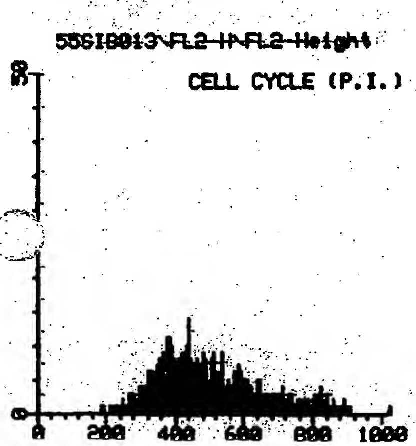
--- Arithmetic Histogram Statistics for 55SIB013

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1622	100.00	16	4.49
1	1.00, 2.64	416	25.65	12	2.07
2	2.64, 9.91	1074	66.21	16	5.19
3	9.91, 118	146	9.00	10	11.86

9



Pos Control
Θ TdT

BECTON
DICKINSON

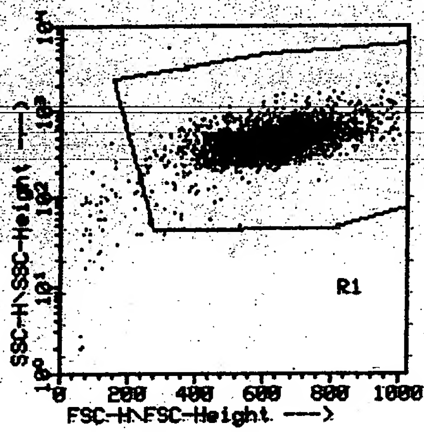
LYSYS II Ver 1.1 2/6/92

DATE: 5-MAY-95

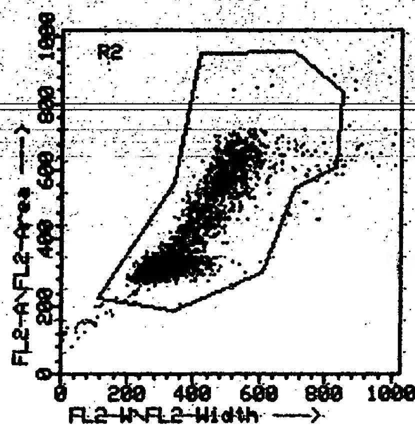
TIME: 15:19:50

SELECTED PREFERENCES: Arithmetic/Linear

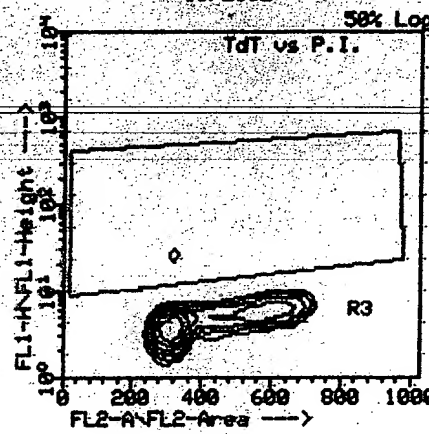
55FL002



55FL002



55FL002



55FL002

----- Region Stats -----

File: 55FL002 Sample: SIBLEY APOP 5

Date: 5/ 5/95 Gate G2= R1ANDR2

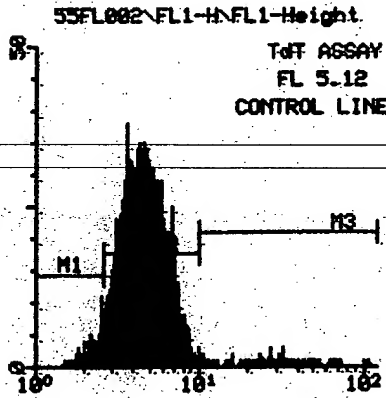
Selected Preference: Arithmetic/Line

Parameters: FL2-A(LIN),FL1-H(LOG)

Total= 3000 Gated= 2684

Rgn	Events	% Gated	% Total
1 R1	2684	100.00	89.47
2 R2	2684	100.00	89.47
3 R3	58	2.16	1.93

55FL002\FL1-H\FL1-Height



55FL002\FL1-H\FL1-Height

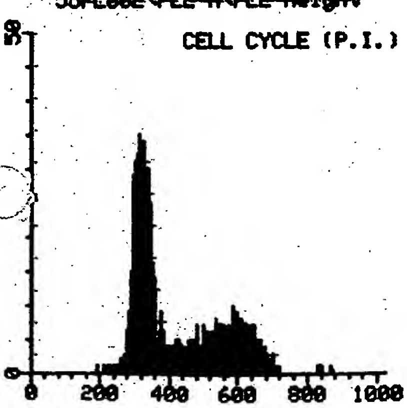
--- Arithmetic Histogram Statistics for 55FL002 -

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	2684	100.00	38	4.49
1	1.00, 2.64	121	4.51	11	2.35
2	2.64, 9.91	2501	93.18	38	4.53
3	9.91, 118	68	2.53	3	27.14

55FL002\FL2-H\FL2-Height



2.5

Positive Control
⊕ TdT

BECTON
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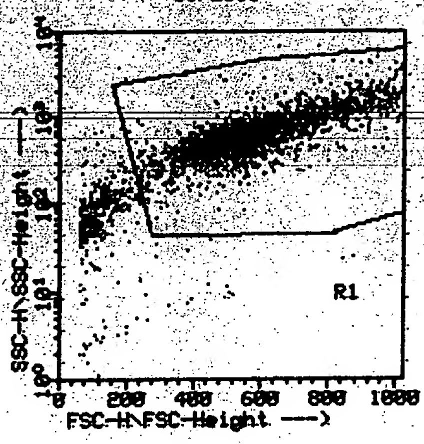
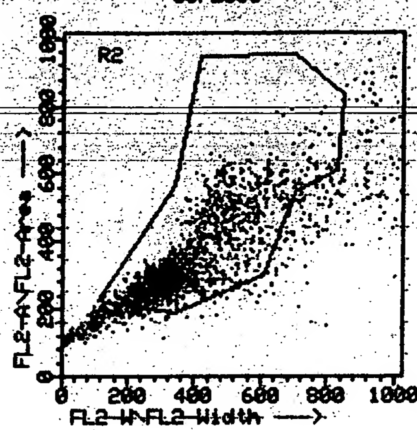
LYSYS II Ver 1.1 2/6/92

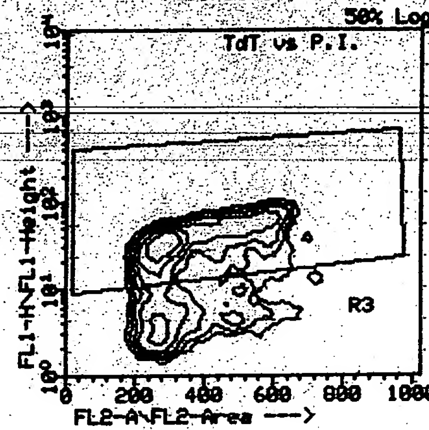
DATE: 5-MAY-95

TIME: 15:21:40

SELECTED PREFERENCES: Arithmetic/Linear







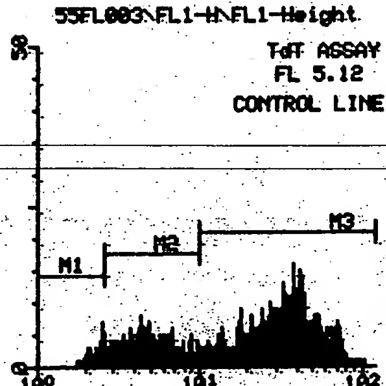
55FL003

----- Region Stats -----

File: 55FL003 Sample: SIBLEY APOP 5
Date: 5/ 5/95 Gate G2= R1ANDR2
Selected Preference: Arithmetic/Line
Parameters: FL2-A(LIN),FL1-H(LOG)
Total= 3000 Gated= 1439

Rgn	Events	% Gated	% Total
1 R1	1439	100.00	47.97
2 R2	1439	100.00	47.97
3 R3	962	66.85	32.07

55FL003\FL1-H\FL1-Height



55FL003\FL1-H\FL1-Height

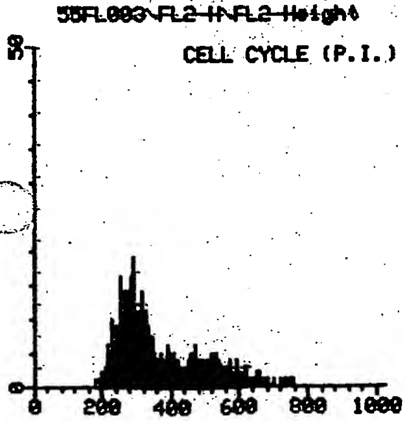
--- Arithmetic Histogram Statistics for 55FL003 -

Selected Preferences: Arithmetic/Linear

Parameter FL1-H FL1-Height Gate G2= R1ANDR2

M	Left,Right	Events	%	Peak	Median
0	1.00, 9910	1439	100.00	16	25.71
1	1.00, 2.64	56	3.89	7	2.44
2	2.64, 9.91	374	25.99	8	4.96
3	9.91, 118	1012	70.33	16	34.29

55FL003\FL2-H\FL2-Height



70.3

5/4/95

Mouse #	Cage #	Group	Day 0				weight	Day 3					weight
1710	AA105283	10^7 899-6	0	0	0								
2075	AA105283	10^7 899-6	0	0	0								
1713	AA105283	10^7 899-6	0	0	0								
2054	AA111331	10^7 899-6	0	0	0								
2057	AA111332	10^7 899-6											
1709	AA104549	10^7 3616											
1723	AA104549	10^7 3616	0	0	0								
2041	AA111334	10^7 3616											
2064	AA111334	10^7 3616											
1859	AA108972	RT Alone	12.5	11.5	9								
1861	AA108972	RT Alone											
1862	AA108972	RT Alone	6	5	4								
1892	AA108964	RT Alone	6	4.5	3								
1863	AA108971	10^7 3616+RT	4.5	4	3.5								
1864	AA108971	10^7 3616+RT	14	9.5	7								
1865	AA108971	10^7 3616+RT	4.5	3.5	1								
1866	AA108971	10^7 3616+RT	4	5	1								
1883	AA108966	10^7 3616+RT	0	0	0								
1884	AA108966	10^7 3616+RT	1	1	1								
1886	AA108966	10^7 3616+RT	0	0	0								
2074	AA108966	10^7 3616+RT	9.5	8.5	6								
2032	AA111326	10^7 3616+RT	6	5	4.5								
2033	AA111326	10^7 3616+RT	5	4	3								
2035	AA111326	10^7 3616+RT	8	7	4.5								
2072	AA111326	10^7 3616+RT	0	0	0								
2036	AA111327	10^7 3616+RT	7	6	3.5								
2037	AA111327	10^7 3616+RT	4	3	1.5								
2039	AA111327	10^7 3616+RT	9	8	5								
2073	AA111327	10^7 3616+RT	4	5	1.5								
1871	AA108969	10^7 899-6+RT	3.5	3.5	2								
1872	AA108969	10^7 899-6+RT											
1873	AA108969	10^7 899-6+RT	5	7	4.5								
1874	AA108969	10^7 899-6+RT	0	0	0								
1880	AA108967	10^7 899-6+RT	0	0	0								
1881	AA108967	10^7 899-6+RT	0	0	0								
18[illegible]	AA108967	10^7 899-6+RT	2.5	2	1								
2[illegible]	AA111329	10^7 899-6+RT	4.5	3.5	2.5								
20[illegible]	AA111329	10^7 899-6+RT	0	0	0								
2046	AA111329	10^7 899-6+RT	4.5	5	2.5								
2047	AA111329	10^7 899-6+RT											
2048	AA111330	10^7 899-6+RT	2.5	2.5	1.5								
2049	AA111330	10^7 899-6+RT	16	13.5	11.5								
2050	AA111330	10^7 899-6+RT	2.5	3.5	2								
2051	AA111330	10^7 899-6+RT	9	8	4.5								

2204			11.5	11	9							
2189			10	11	9							
2190			12	10	8							
2191			13	9	8							
2192			12	10.5	9							
2201			6.5	6.5	4.5							
2202			7	7.5	5							
2203			7	7.5	4.5							

LOGSHEETS 5/8

1710	AA105283	10^7 899-6	<10 d35	0	23.7		
2075	AA105283	10^7 899-6	<10 d42	0	23.0		
1713	AA105283	10^7 899-6	<10 d14	0	22.2		
~~2054~~	~~AA111331~~	~~10^7 899-6~~	~~<10 d49~~	~~0~~	23.4		
1709	AA104549	10^7 3616	<10 d31				
1723	"	"		0	24.3		
1859	AA108972	RT Alone		12.5x11x10	24.0		
1862	AA108972	RT Alone		5.5.3	25.1		
1892	AA108964	RT Alone		5x4x2	24.4		
2189	AA113941	RT Alone		10x11.5x8	22.0		
2190	AA113941	RT Alone		11.5x8.5x7	18.2		
2191	AA113941	RT Alone		12.5x8x6.5	18.6		
2192	AA113941	RT Alone		12x10x8	22.0		
2201	AA113944	RT Alone		8.5x10x8	22.6		
~~2202~~	~~AA113944~~	~~RT Alone~~		~~8.5x10x7~~	~~21.3~~		
2203	AA113944	RT Alone		8x7x6	24.3		
2204	AA113944	RT Alone		12.5x12.5x10	23.1		
1863	AA108971	10^7 3616+RT		5.5x4x3	24.7		
1864	AA108971	10^7 3616+RT		13.5x10x6.5	27.6		
1865	AA108971	10^7 3616+RT	<10 d38	4x3x1	27.0		
1866	AA108971	10^7 3616+RT	<10 d31	0	26.3		
1883	AA108966	10^7 3616+RT	<10 d24	0	22.6		
1884	AA108966	10^7 3616+RT	<10 d56	2x2x1.5	23.9		
1886	AA108966	10^7 3616+RT	<10 d31	0	21.5		
2074	AA108966	10^7 3616+RT		9.5x9x6	17.0		
2032	AA111326	10^7 3616+RT		6x7.5x4.5	22.1		
2033	AA111326	10^7 3616+RT		4x4x2.5	25.0		
2035	AA111326	10^7 3616+RT		7x8.5x5	22.2		
2072	AA111326	10^7 3616+RT	<10 d21	~~000~~ 0	24.7		
2036	AA111327	10^7 3616+RT		~~6x2.5x4.5~~	~~22.1~~		
2037	AA111327	10^7 3616+RT	<10 d52	3x2x1	25.3		
2039	AA111327	10^7 3616+RT		8.5x9x5.5	17.6		
2073	AA111327	10^7 3616+RT		4x4x1	20.6		
				6.5x6x3	22.8		
1871	AA108969	10^7 899-6+RT	<10 d66	3.5x3.5x2	24.0		
1873	AA108969	10^7 899-6+RT		6x6x4	24.6		
1874	AA108969	10^7 899-6+RT	<10 d52	0	27.8		
1880	AA108967	10^7 899-6+RT	<10 d14	0	21.7		
1881	AA108967	10^7 899-6+RT	<10 d42	0	26.4		
1882	AA108967	10^7 899-6+RT	<10 d24	0	24.0		
2044	AA111329	10^7 899-6+RT		4.5x4x3	23.8		
2045	AA111329	10^7 899-6+RT	<10 d21	0	27.3		
2046	AA111329	10^7 899-6+RT		4.5x4x2	25.0		
2048	AA111330	10^7 899-6+RT	<10 d38	2x2.5x1	15.9		
2049	AA111330	10^7 899-6+RT		18.5x14x13.5	25.2		
2050	AA111330	10^7 899-6+RT	<10 d31	3x2x1.5	23.9		
2051	AA111330	10^7 899-6+RT		7x8x3.5	24.0		

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1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
~~1709~~	AA104549	10^7 3616	<10 d31				
1723				0			
1859	AA108972	RT Alone	11.5x10x10.5				
1862	AA108972	RT Alone	4.5x5x3.5				
1892	AA108964	RT Alone	4.5x3.5x3				
2189	AA113941	RT Alone	10.5x11x9				
2190	AA113941	RT Alone	11.5x8x8.5				
2191	AA113941	RT Alone	10.5x7x6.5				
2192	AA113941	RT Alone	11x12x9				
2201	AA113944	RT Alone	10x9.5x9				
2202	AA113944	RT Alone	7.5x7x6.5				
2203	AA113944	RT Alone	8.5x10x7				
2204	AA113944	RT Alone	11.5x11x9				
1863	AA108971	10^7 3616+RT		4x5x3			
1864	AA108971	10^7 3616+RT		15x12x8			
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	2x2x1.5			
1886	AA108966	10^7 3616+RT	<10 d31	~~3x4x7~~ 0			
2074	AA108966	10^7 3616+RT		8x9x4			
2032	AA111326	10^7 3616+RT	~~5x7x4.5~~	5x7x4.5			
2033	AA111326	10^7 3616+RT		4x4x2.5			
2035	AA111326	10^7 3616+RT		8x6x5			
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		5.5x6.5x3			
2037	AA111327	10^7 3616+RT	<10 d52	3x2.5x1.5			
2039	AA111327	10^7 3616+RT		10x8x6			
2073	AA111327	10^7 3616+RT		4x3x1			
1871	AA108969	10^7 899-6+RT	<10 d66	3x4x2.5			
1873	AA108969	10^7 899-6+RT		6x5x3			
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		5x4x4			
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		4x3.5x2.5			
2048	AA111330	10^7 899-6+RT	<10 d38	1.5x1x1			
2049	AA111330	10^7 899-6+RT		18x13x11			
2050	AA111330	10^7 899-6+RT	<10 d31	3x2x1			
2051	AA111330	10^7 899-6+RT		6x8x4.5			

OVER

5/15/95

1710	AA105283	10^7 899-6	<10 d35	0	0	0	23.8
2075	AA105283	10^7 899-6	<10 d42	0	0	0	22
1713	AA105283	10^7 899-6	<10 d14	0	0	0	20.8
2054	AA111331	10^7 899-6	<10 d49	0	0	0	22.4
1709	AA104549	10^7 3616	<10 d31	0	0	0	25.3
23							
1859	AA108972	RT Alone		12	14	10	24.5
1862	AA108972	RT Alone		5.5	9	3	24.4
1892	AA108964	RT Alone		5	5	3	23.5
2189	AA113941	RT Alone		11	12	8	22.0
2190	AA113941	RT Alone		12	9.5	7	17.8
2191	AA113941	RT Alone		12	8.5	6	19.2
2192	AA113941	RT Alone		12	11	9	20.3
2201	AA113944	RT Alone		11	12	9	22.2
2202	AA113944	RT Alone		6.5	6.5	5.5	24.2
2203	AA113944	RT Alone		11.5	10	8.5	20.4
2204	AA113944	RT Alone		12	11.5	7	19.9
1863	AA108971	10^7 3616+RT		5	4	2	23.3
1864	AA108971	10^7 3616+RT		14	13	7	25.4
1865	AA108971	10^7 3616+RT	<10 d38	0	0	0	25.6
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	25.2
1883	AA108966	10^7 3616+RT	<10 d24	0	0	0	22.1
1884	AA108966	10^7 3616+RT	<10 d56	0	0	0	23.9
1886	AA108966	10^7 3616+RT	<10 d31	0	0	0	21.4
2074	AA108966	10^7 3616+RT		10	9.5	4	16.8
2032	AA111326	10^7 3616+RT		8	7	5.5	23.2
2033	AA111326	10^7 3616+RT		4.5	4	1.5	25.1
2035	AA111326	10^7 3616+RT		0	0	0	24.4
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	4	23.3
2036	AA111327	10^7 3616+RT		6	6.5	3	23.8
2037	AA111327	10^7 3616+RT	<10 d52	5	3.5	1.5	25.2
2039	AA111327	10^7 3616+RT		11	9	6	16.8
2073	AA111327	10^7 3616+RT		0	0	0	20.4
1871	AA108969	10^7 899-6+RT	<10 d66	7	6	2.5	23.9
1873	AA108969	10^7 899-6+RT		5	5	3	23.3
1874	AA108969	10^7 899-6+RT	<10 d52	0	0	0	27.9
1880	AA108967	10^7 899-6+RT	<10 d14	0	0	0	21.2
1881	AA108967	10^7 899-6+RT	<10 d42	0	0	0	25.1
1882	AA108967	10^7 899-6+RT	<10 d24	6	5	1.5	23.9
2044	AA111329	10^7 899-6+RT		7	6	4.5	24.3
2045	AA111329	10^7 899-6+RT	<10 d21	3.5	3	2	25.4
2046	AA111329	10^7 899-6+RT		8	0	0	25.4
2048	AA111330	10^7 899-6+RT	<10 d38	0	0	0	16.2
2049	AA111330	10^7 899-6+RT		21.5	15	14	25.3
2050	AA111330	10^7 899-6+RT	<10 d31	0	0	0	23.8
2051	AA111330	10^7 899-6+RT		8	10	5.5	24.6

113945 L 2089 8.5 9.5 7.5 21.3
R 2090 9 8.5 7.5 22
LR

5/18/95

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
22							
1859	AA108972	RT Alone		12.5	14	10.5	
1862	AA108972	RT Alone		5	5	3.5	
1892	AA108964	RT Alone		4.5	5	2.5	
2189	AA113941	RT Alone		11	11.5	8	
2190	AA113941	RT Alone		9	12	6.5	
2191	AA113941	RT Alone		8	11	4	
2192	AA113941	RT Alone		11	11	7	
2201	AA113944	RT Alone		11	11	7	
2202	AA113944	RT Alone		7	7	5	
2203	AA113944	RT Alone		11.5	12	9	
2204	AA113944	RT Alone		12	12	8.5	
1863	AA108971	10^7 3616+RT		4	5	2	
1864	AA108971	10^7 3616+RT		14	11.5	6	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0	0	0	
1883	AA108966	10^7 3616+RT	<10 d24	0	0		
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	~~12~~ 0	~~10~~ 0	~~6~~ 0	
2074	AA108966	10^7 3616+RT		13	10	6	
2032	AA111326	10^7 3616+RT		6.5	8	6	
2033	AA111326	10^7 3616+RT		4	3.5	2	
2035	AA111326	10^7 3616+RT		0			
2072	AA111326	10^7 3616+RT	<10 d21	8.5	8.5	6	
2036	AA111327	10^7 3616+RT		6	7	2.5	
2037	AA111327	10^7 3616+RT	<10 d52	4	4	2	
2039	AA111327	10^7 3616+RT		12	10	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	6	5.5	2.5	
1873	AA108969	10^7 899-6+RT		5	5	3	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	0			
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		6.5	6	4	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3.5	3.5	2	
2048	AA111330	10^7 899-6+RT	<10 d38	3.5	3	1	
2049	AA111330	10^7 899-6+RT		22000			
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		7.5	10	5	

113945 2206 9.5 9 7.5 CONTROLS
2207 11 11.5 9

AVER

5/22/95

1710	AA105283	10^7 899-6	<10 d35	0			23.2
2075	AA105283	10^7 899-6	<10 d42	0			22.3
~~1713~~	~~AA105283~~	~~10^7 899-6~~	~~<10 d14~~	0			
2054	AA111331	10^7 899-6	<10 d49	0			
1709	AA104549	10^7 3616	<10 d31	0			
1859	AA108972	RT Alone		14	13.5	10	25
1862	AA108972	RT Alone		5	7	4	24.4
1892	AA108964	RT Alone		5	4	3	23.8
2189	AA113941	RT Alone		10.5	10	8	20.9
died ~~2190~~ 2198	AA113941	RT Alone		11	8	6.5	18.2
drown ~~2191~~	AA113941	RT Alone					
2192	AA113941	RT Alone		11	9	7	22.2
2201	AA113944	RT Alone		10.5	10.5	8	20.3
2202	AA113944	RT Alone		6.5	5	4	24
2203	AA113944	RT Alone		10	10	7	20.6
2204	AA113944	RT Alone		11	11.5	8.5	21.1
1863	AA108971	10^7 3616+RT		4	3	3	23.8
1864	AA108971	10^7 3616+RT		13.5	12	7	25.3
1865	AA108971	10^7 3616+RT	<10 d38	0			26.4
1866	AA108971	10^7 3616+RT	<10 d31	0			26.2
1883	AA108966	10^7 3616+RT	<10 d24	0			22.2
1884	AA108966	10^7 3616+RT	<10 d56	2	1	1	24.2
1886	AA108966	10^7 3616+RT	<10 d31	0			26.2
2074	AA108966	10^7 3616+RT		16	11	7	27
2032	AA111326	10^7 3616+RT		7.5	5.5	4.5	23.3
2033	AA111326	10^7 3616+RT		4	3	2.5	25.7
2035	AA111326	10^7 3616+RT		9.5	7	6	23.9
2072	AA111326	10^7 3616+RT	<10 d21	0		4.	25.5
2036	AA111327	10^7 3616+RT		6	5	1.5	26.6
2037	AA111327	10^7 3616+RT	<10 d52	3	3		26
2039	AA111327	10^7 3616+RT		10	8.5	6	18
2073	AA111327	10^7 3616+RT		0			21.6
1871	AA108969	10^7 899-6+RT	<10 d66	5	4	2	25.4
1873	AA108969	10^7 899-6+RT		7	5	4	24.6
1874	AA108969	10^7 899-6+RT	<10 d52	0			28.3
1880	AA108967	10^7 899-6+RT	0 d14	0			22.0
1881	AA108967	10^7 899-6+P	0 d42	6	5	3.5	26.6
1882	AA108967	10^7 899-6	0 d24	0			24.2
2044	AA111329	10^7 899-6+R		5	6.5	4.5	25.9
2045	AA111329	10^7 899-6+RT	<.0 d21	0			18.7
2046	AA111329	10^7 899-6+RT		3.5	3	2	24.9
2048	AA111330	10^7 899-6+RT	<10 d38	0			15.9
~~2049~~	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	5	3	2	25.6
2051	AA111330	10^7 899-6+RT		8.5	10.5	7.5	24.7

5/23/95

IN VIVO TNF PRODUCTION

Group	Mouse	Time	Tum weight	Vol Buffer	[TNF]	[TNF/tumor	TNF/mg-tumor
2x10^7 PFU 899-6	2370	3d	.45				
"	2371	3d	.26				
"	2372	3d	.37				
"	2373	3d	.44				
2x10^7 899-6	2374 ~~2386~~	10 d	.13				
"	2375 ~~2387~~	10 d	.54				
"	2376 ~~2388~~	10 d	.61				
"	2377 ~~2389~~	10 d	.23				
Control	2386	10d	2.41				
"	2387	10d	.35				
"	2388	10d	.47				
"	~~2389~~	~~10d~~					
2x10^7 3616	2390	3d	0.44				
"	2391	3d	1.23				
"	2392	10d	.24				
"	2393	10d	.69				

INDUCTION- in vivo							
Mouse	Treatment	Tumor Wt	Vol Buffer	Reading	[TNF]pg/ml	[TNF]/tumor	[TNF]/n
2370	3d 899-6	0.45	1	0.679	331	480	1.0661
2371	3d 899-6	0.26	1	0.708	348	439	1.687
2372	3d 899-6	0.37	1	1.326	747	1023	2.7641
2373	3d 899-6	0.44	1	1.42	811	1168	2.6553
2374	10d 899-6	0.13	1	0.352	149	168	1.2938
2375	10d 899-6	0.54	1	0.444	197	304	0.5629
2376	10d 899-6	0.61	1	0.274	110	177	0.2897
2377	10d 899-6	0.23	1	0.323	134	165	0.717
2386	10d Control	2.41	1	2.021	1246	4249	1.7632
2387	10d Control	0.35	1	1.823	1099	1484	4.2401
2388	10d Control	0.47	1	2.498	1612	2370	5.0426
2390	3d 3616	0.44	1	0.37	158	228	0.5176
2391	3d 3616	1.23	1	0.515	236	527	0.4286
2392	10d 3616	0.24	1	0.374	160	199	0.8279
2393	10d 3616	0.69	1	0.756	377	637	0.9234

$$529.72 \times X^{1.2158}$$

$$y = .005863 \times X^{.8183911}$$

PLATE #

DATE:

O.D. LIMIT: 3.000

TIME:

WAVELENGTH: 450nm

READ MODE: OPTICAL DENSITY

AUTO MIX

OPTICAL DENSITY

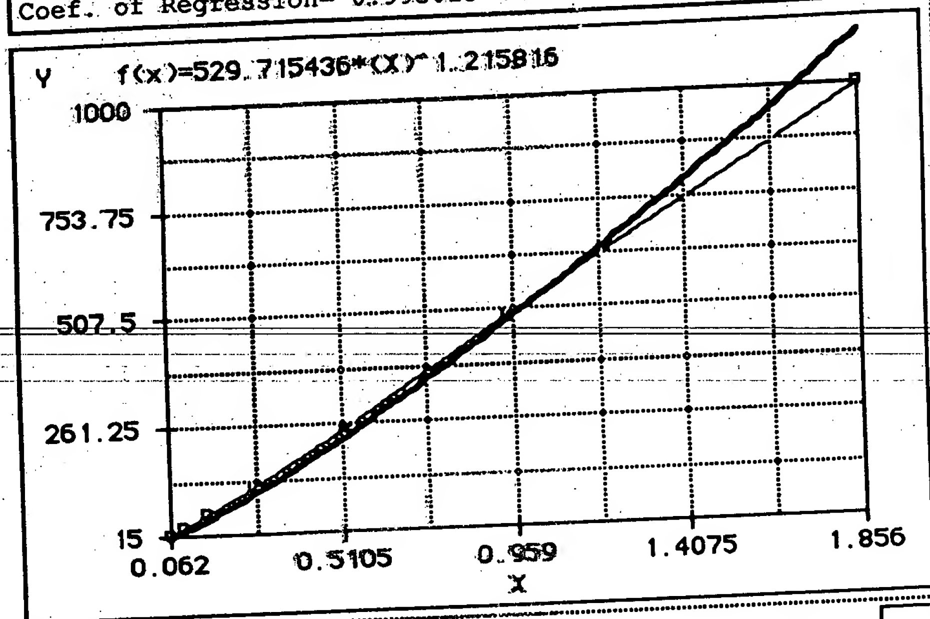
1	2	3	4	5	6	7	8	9	10	11	12

Curve Fit-Plot © 1990 JCA

Modif./Inv. DATA:

X	Y
0.062	15.13
0.095	31.13
0.158	62.5
0.282	125
0.518	250
0.943	500
1.856	1000

• EXPONENTIAL FIT •
Y= 529.715436* (X)^1.215816
Coef. of Regression= 0.995013 For 7 Data Pairs



INITIALS Y.K. (5/26)
DATE 6/9/95

SUBJECT • Untilted ; [I: X<>Y]

CPU Time: 13 Secs.

5/25/95

1710	AA105283	10^7 899-6	<10 d35	0			
2075	AA105283	10^7 899-6	<10 d42	0			
1713	AA105283	10^7 899-6	<10 d14	0			
2054	AA111331	10^7 899-6	<10 d49	0			
~~1709~~	AA104549	10^7 3616	<10 d31	0			
1723							
1859	AA108972	RT Alone		13	13	11	
1862	AA108972	RT Alone		4.5	4	3	
1892	AA108964	RT Alone		4	4.5	2.5	
2189	AA113941	RT Alone		7	9.5	5.5	
2190	AA113941	RT Alone		9.5	10.5	7	
~~2191~~	~~AA113941~~	RT Alone					
2192	AA113941	RT Alone		9	10	6.5	
2201	AA113944	RT Alone		10	11	8.5	
2202	AA113944	RT Alone		5.5	4	2.5	
2203	AA113944	RT Alone		9	10	7.5	
2204	AA113944	RT Alone		11	11.5	7.5	
1863	AA108971	10^7 3616+RT		3.5	3	3	
1864	AA108971	10^7 3616+RT		13	12	7.5	
1865	AA108971	10^7 3616+RT	<10 d38	0			
1866	AA108971	10^7 3616+RT	<10 d31	0			
1883	AA108966	10^7 3616+RT	<10 d24	0			
1884	AA108966	10^7 3616+RT	<10 d56	0			
1886	AA108966	10^7 3616+RT	<10 d31	0			
2074	AA108966	10^7 3616+RT		10	14	6	
2032	AA111326	10^7 3616+RT		6.5	7	5	
2033	AA111326	10^7 3616+RT		4	3	2	
2035	AA111326	10^7 3616+RT		7	8.5	5	
2072	AA111326	10^7 3616+RT	<10 d21	0			
2036	AA111327	10^7 3616+RT		6	4.5	3.5	
2037	AA111327	10^7 3616+RT	<10 d52	0			
2039	AA111327	10^7 3616+RT		8	9	5	
2073	AA111327	10^7 3616+RT		0			
1871	AA108969	10^7 899-6+RT	<10 d66	0			
1873	AA108969	10^7 899-6+RT		6	5	3.5	
1874	AA108969	10^7 899-6+RT	<10 d52	0			
1880	AA108967	10^7 899-6+RT	<10 d14	0			
1881	AA108967	10^7 899-6+RT	<10 d42	6	7.5	4	
1882	AA108967	10^7 899-6+RT	<10 d24	0			
2044	AA111329	10^7 899-6+RT		8	6.5	5.5	
2045	AA111329	10^7 899-6+RT	<10 d21	0			
2046	AA111329	10^7 899-6+RT		3	4.5	1.5	
2048	AA111330	10^7 899-6+RT	<10 d38	0			
2049	AA111330	10^7 899-6+RT					
2050	AA111330	10^7 899-6+RT	<10 d31	0			
2051	AA111330	10^7 899-6+RT		13	9	9	

5/29/95 Control
2354 15.5 14.5 11.5 23.5
55 20 15.5 12 21.2
56 15.5 11 8.5 23.8
57 13 16 10 21.0

3616+RT
2032 8 6.5 7 23.9
33 3 3.5 2 25.8
2072 0 24.7
35 9 8 6 23.8

1	2	3	4	5	6	7	8	9

RT
2342 14 14 6 20.9
43 12.5 10.5 8 22.3
44 10 10 8 20.6
45 12 10 7 21.2

RT
2350 14 11.5 8 21.7
51 9.5 7 6 22.7
52 10 9 7 23.6
53 14 9 8 20.6

RT
2189 9 9.5 7 20.6
90 7 6 4 18.4
92 9.5 9 5.5 24.1

RT
2201 10 10 9 19.3
02 2.5 2.5 1 26.6
03 11.5 11 6 20.0
04 12 11 7.5 23.7

RT
1859 12 13 11.5 26.3
1892 4.5 3 2.5 24.3
1862 5 4.5 4.5 25.1

RT
2346 13.5 10.5 10 19.2
47 12 10 7.5 23.0
48 16 10 9 18.6
49 9 11.5 7 22

CONTROL
2206 17.5 20 16.5 24.9
07 13 14.5 11.5 24.9

CONTROL
2358 16 15.5 14 20.9
59 16 15.5 11.5 20.7
60 17.5 12 11 23.4
61 13 16 10 22

3616+RT
2036 5.5 4.5 4 25.1
37 0 26.5
2073 0 21.3
39 8 7 4 21.2

3616+RT
1883 0 21.6
84 0 23.9
2074 15.5 10 7 16.9
86 0 21.3

3616+RT
1863 4 4 3.5 24.2
64 13 11 7 22.6
65 0 26.3
66 0 25.3

3616
1723 0

899-6
2054 0 23.2
2075 0 24.2
1713 0 22
1710 0 24.4

899+RT
1880 0 21.4
81 8 5 4 27
82 0 24

899+RT
2048 0 14.6
50 0 25.3
51 14 10 9 25.1

~~RT~~ CONT'D
2354 17 17.5 11
55 22 17.5 11.5
56 16 14.5 8
57 16.5 ~~8~~13 9

6/1/95

3616 + RT
2032 8 8.5 6
33 4 3 2.5
2072 [redacted] 0
35 9 7.5 6

RT
2342 15.5 13.5 9.5
2343 12 9 7
2344 13 11 7.5
2345 7.5 10 7.5

RT
2350 17 10 8.5
2351 11 8 7
2352 10 8 7
2353 14 9.5 7

RT
2189 10 10 7
2190 6 6 3.5
2192 9.5 10 5

RT
2201 9 11 8
2202 0
03 9.5 9.5 5
04 11 11.5 7

RT
[redacted]

RT
2346 14 12 8.5
2347 12 11 8
2348 15 10 9
2349 11.5 10 7

CONTROL
2358 19.5 18 14
59 18.5 17.5 12
60 15 18 12
61 15.5 17.5 12

CONTROL
2206 20 22 18
2207 14.5 14.5 13.5

3616 + RT
[redacted] 5 4 3
[redacted] 5 10.5 7
[redacted] 0
[redacted] 0

3616 + RT
[redacted] 0
[redacted] 2 1 1
?sac ⇒ 2074 10 14 5.5
[redacted] 0

3616 + RT
2036 6 5 4
37 0
2073 0
2039 8 7 4

899-6 + RT
?sac ⇒ 2048 0
2050 6 4 1
2051 12 15 9

899 + RT
[redacted] 0
[redacted] 7 9.5 6
[redacted] 0

899 ~~+RT~~
2054 0
2075 0
[redacted] 0
[redacted] 0

899-6 + RT
2044 8 8.5 7
45 dead
46 0

The University of Chicago Departmental Purchase Order

Purchase Order Number **Z904416**

THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name

PHAR MINGEN
10975 Torreyana Rd
San Diego, CA 92121

City ____ State ____ Zip Code ____

Payment Terms ______ Delivery charge? [X] Yes [ ] No

Telephone No. 1-800-848-6227 FAX No. 619-792-5238

Ship To

THE UNIVERSITY OF CHICAGO — Dept. Code: ______

Department: Dept Radiation Oncology
Attn: Greg Sibley, MD — Room: 6-03
5830 S. Ellis Ave
City: Chicago State: IL Zip Code: 60637

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

Authorized Signature ______ Payroll No. ______
Print Name ______ Ext. ______
Account Code ______ Date ______

Order placed by phone? [ ] No [X] Yes
Order placed with (name): Darren — Date: 6/2/95
Order placed by (name): Greg Sibley — Phone #: 2-0294

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1	18641-D Purified Mouse Anti-human TNFα	$250	
		(Backordered 1-2 wks)	$ 20	

DEPARTMENT COPY — **NOT VALID IF TOTAL EXCEEDS $500.00** — ORDER TOTAL $270

BACK ORDERS ARE NOT ALLOWED ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

The University of Chicago Departmental Purchase Order

Purchase Order Number **Z904417**

THIS NUMBER MUST APPEAR ON ALL PACKAGES, INVOICES AND PACKING SLIPS

NOT VALID IF TOTAL EXCEEDS $500.00.
Not to be used for purchase of travel, hazardous or radioactive materials, controlled substances, vehicle rental or other restricted items.

Vendor Name
VECTOR LABORATORIES
30 INGOLD RD
BURLINGAME, CA 94010
City State Zip Code

Payment Terms ____ Delivery charge? ☐ Yes ☐ No
Telephone No. 800-227-6666 FAX No. 415-697-0339

Ship To
THE UNIVERSITY OF CHICAGO
Dept. Code: ____
Dept Radiation Oncology
Department
Greg Sibley, MD
G-03
Room
5830 S. Ellis Ave
Chicago IL 60637
City State Zip Code

Authorized Signature ____ Payroll No. ____
Print Name ____ Ext. ____
Account Code ____ Date ____

Order placed by phone? ☐ No ☒ Yes
Virginia — 6/2/95
Order placed with (name) — Date
Greg Sibley — 2-0294
Order placed by (name) — Phone #

IMPORTANT INFORMATION

Not to be used for purchase of hazardous or radioactive materials; hazardous waste removal; animals; controlled substances (narcotics, ethyl alcohol, dangerous drugs); goods or services which should be obtained from campus departments as stated in University policies & procedures; chaining, where two or more orders, each under $500.00, are used to exceed the restriction of $500.00 for one purchase; travel expenses; any services performed by an individual which may be reportable to IRS as wages on form 1099; or goods or services that require payment to accompany the order.

1. ALL items must be filled out by REQUESTING department.
2. Order MUST be typewritten.
3. Original copy is for ISSUING department; send copy to vendor if required. Second copy should be sent to the Purchasing Department.
4. Vendor and Ship To must have complete addresses.
5. Department Code MUST be filled in with 4-digit department code from listing already furnished.
6. Only ONE account code is allowed per order.

QTY	UNIT	DESCRIPTION	NET UNIT PRICE	ITEM TOTAL
1	1 Kit	PK-4002 Peroxidase vectastain ABC - Mouse IgG	$185.00	
			6.25	

ORDER TOTAL $191.25

DEPARTMENT COPY

NOT VALID IF TOTAL EXCEEDS $500.00
BACK ORDERS ARE NOT ALLOWED
ON THIS PURCHASE ORDER

Form ZDPO 100 07/94

6/5/95 J-013

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207 L	AA113945	Control		18	16	11		28.4
2354	AA117027	Control ϕ		22	19	9		23.3
2356	AA117027	Control R		18	16.5	7		23.7
2357	AA117027	Control LR		17	14	10		19.6
2359	AA117028	Control L		22	18	11		20.5
2360	AA117028	Control R	18		18	12		24.6
2361	AA117028	Control LR		21	20	11		24.8
1710 LR	AA105283	10^7 899-6	<10 d35	ϕ	ϕ	ϕ		24.7
2075 ϕ	AA105283	10^7 899-6	<10 d42	ϕ	ϕ	ϕ		24.5
1713 R	AA105283	10^7 899-6	<10 d14	ϕ	ϕ	ϕ		21.5
2054 LR	AA~~111331~~ 105283	10^7 899-6	<10 d49	ϕ	ϕ	ϕ		22.5
1723	AA104549	10^7 3616	<10 d28 face	ϕ	ϕ	ϕ		26.5
1859	AA108972	RT Alone ϕ		15	14	10		24.8
1862	AA108972	RT Alone LR		5	5	3		25.1
1892	AA1089~~64~~ 72	RT Alone R		4	4	2		24.1
2189 ϕ	AA113941	RT Alone		10	10	4.5		24.0
2190 L	AA113941	RT Alone		5	5	2.5		20.9
2192 LR	AA113941	RT Alone		9	9	3		25.2
2201 ϕ	AA113944	RT Alone		8	8	3		19.6
2202 L	AA113944	RT Alone		ϕ	ϕ	ϕ		27.0
2203 R	AA113944	RT Alone		11	11	4.5		20.8
2204 LR	AA113944	RT Alone		12	11.5	7		26.6
2342 ϕ	AA117024	RT Alone		14	12	5		22.1
2343 L	AA117024	RT Alone		11.5	9	3.5		23.5
2344 R	AA117024	RT Alone		12.5	9	5		22.4
2345 LR	AA117024	RT Alone		15	8.5	5		22.9
2346 ϕ	AA117025	RT Alone		15	10	6		20.0
2347 L	AA117025	RT Alone		11.5	11.5	7		23.9
2348 R	AA117025	RT Alone		16	11	7		19.4
2349 LR	AA117025	RT Alone		12	11	5		21.7
2350	AA117026	RT Alone ϕ		16	10	6		21.3
2351	AA117026	RT Alone L		10	8	4		23.4
2352	AA117026	RT Alone R		10	10	4		21.7
2353	AA117026	RT Alone LR		13	9	1		22.1

Tuesday: Δ Cage for biohazard animals Done ✓

1863 φ	AA108971	10^7 3616+RT		4	3	2		24.1
~~1864~~ L	~~AA108971~~	~~10^7 3616+RT~~		11	10	3		25.5
1865 R	AA108971	10^7 3616+RT	<10 d38	φ	φ	φ		26.4
~~1866~~ LR	~~AA108971~~	~~10^7 3616+RT~~	~~<10 d31~~	φ	φ	φ		25.6
1883 φ	AA108966	10^7 3616+RT	<10 d24	φ	φ	φ		23.1
1884 L	AA108966	10^7 3616+RT	<10 d56	φ	φ	φ		23.4
1886 LR	AA108966	10^7 3616+RT	<10 d31	φ	φ	φ		19.8
2074 R	AA108966	10^7 3616+RT		11	10	3		14.7
2032 φ	AA111326	10^7 3616+RT		9	9	5		25.0
2033 L	AA111326	10^7 3616+RT		4	3	2		26.2
2035 LR	AA111326	10^7 3616+RT		10	7	3		25.1
2072 R	AA111326	10^7 3616+RT	<10 d21	φ	φ	φ		25.7
2036 φ	AA111327	10^7 3616+RT		7	5	3		24.8
2037 L	AA111327	10^7 3616+RT	<10 d52	2	2	2		27.2
2039 LR	AA111327	10^7 3616+RT		8	8	3		22.1
2073 R	AA111327	10^7 3616+RT		φ	φ	φ		21.7
1871 φ	AA108969	10^7 899-6+RT	<10 d66	φ	φ	φ		24.8
1873 R	AA108969	10^7 899-6+RT		5.5	5	2		25.1
1874 LR	AA108969	10^7 899-6+RT	<10 d52	φ	φ	φ		28.5
1880	AA108967	10^7 899-6+RT	<10 d14 L	φ	φ	φ		25.1
1881	AA108967	10^7 899-6+RT	<10 d42 R	8	9	3		27.5
1882	AA108967	10^7 899-6+RT	<10 d24 LR	φ	φ	φ		21.7
2044 φ	AA111329	10^7 899-6+RT		8	8	5		24.7
2046 R	AA111329	10^7 899-6+RT	<10 d77	φ	φ	φ		25.7
2048	AA111330	10^7 899-6+RT	<10 d38 φ	φ	φ	φ		13.5
2050	AA111330	10^7 899-6+RT	<10 d31 R	2	2	1		25.4
2051	AA111330	10^7 899-6+RT	LR	18	13	5		26.3

6/8/95

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control		15	16	14		
2354	AA117027	Control	sac	23	22	13		
2356	AA117027	Control		19.5	17	11		
2357	AA117027	Control		18	15	12		
2359	AA117028	Control	sac	23	20	15		
2360	AA117028	Control	sac	18	17	14.5		
2361	AA117028	Control	sac	22	23	16.5		
1710	AA105283	10^7 899-6	<10 d35	0				
2075	AA105283	10^7 899-6	<10 d42	0				
1713	AA105283	10^7 899-6	<10 d14	8				
2054	AA111331	10^7 899-6	<10 d49	0				
1723	AA104549	10^7 3616	<10 d28 face	0				
1859	AA108972	RT Alone	Sacid ill	12	12	13		
1862	AA108972	RT Alone		4.5	6	4		
1892	AA108964	RT Alone		3.5	5	2.5		
2189	AA113941	RT Alone		+9.5	10	6.5		
2190	AA113941	RT Alone		5	4.5	2.5		
2192	AA113941	RT Alone		9.5	8	4		
2201	AA113944	RT Alone		8.5	7	3.5		
2202	AA113944	RT Alone		0				
2203	AA113944	RT Alone		10	9	5		
2204	AA113944	RT Alone		13	10.5	8.5		
2342	AA117024	RT Alone		12	10.5	6.5		
2343	AA117024	RT Alone		12	10	7		
2344	AA117024	RT Alone		15	12.5	8		
2345	AA117024	RT Alone		+10.5	8.5	5.5		
2346	AA117025	RT Alone		13	11	9		
2347	AA117025	RT Alone		12	10.5	9		
2348	AA117025	RT Alone		14.5	10	8		
2349	AA117025	RT Alone		11	9.5	5		
2350	AA117026	RT Alone		+14.5	10.5	7		
2351	AA117026	RT Alone		10	8	7		
2352	AA117026	RT Alone		9.5	8	6.5		
2353	AA117026	RT Alone		13.5	8.5	7		

17
7
114
x18
952
1190

16
7
112
x15
160
1120

1863	AA108971	10^7 3616+RT		5	4	2.5		
1864	AA108971	10^7 3616+RT		11.5	9.5	6.5		
1865	AA108971	10^7 3616+RT	<10 d38	0				
1866	AA108971	10^7 3616+RT	<10 d31	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	0				
1886	AA108966	10^7 3616+RT	<10 d31	0				
2074	AA108966	10^7 3616+RT		14	10	6		
2032	AA111326	10^7 3616+RT		8	4.5	8		
2033	AA111326	10^7 3616+RT		4	3.5	2		
2035	AA111326	10^7 3616+RT		9	8	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		6	5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		~~4~~7	6	2.5		
2073	AA111327	10^7 3616+RT		0				
1871	AA108969	10^7 899-6+RT	<10 d66	0				
1873	AA108969	10^7 899-6+RT		6	5	4		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	8.5	10	6.5		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		8.5	7.5	6		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
2048	AA111330	10^7 899-6+RT	<10 d38 sac	wt loss				
2050	AA111330	10^7 899-6+RT	<10 d31	0				
2051	AA111330	10^7 899-6+RT		21.5	16	10		

2209 AA113946 10^7 3616 8 6.5 7.5
10 impl 5/3 inj 5/8 7.5 6.5 5.5
11 10 7 6
12 8 8.5 6

6/12/95

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control		21	19	9		23.3
2354	AA117027	Control						
2356	AA117027	Control	dead					
2357	AA117027	Control	dead					
2359	AA117028	Control						
2360	AA117028	Control						
2361	AA117028	Control						
1710	AA105283	10^7 899-6	<10 d35	0				
2075	AA105283	10^7 899-6	<10 d42	0				
1713	AA105283	10^7 899-6	<10 d14	0				
2054	AA111331	10^7 899-6	<10 d49	0				
1723	AA104549	10^7 3616	<10 d28 face	0				
1859	AA108972	RT Alone						
1862	AA108972	RT Alone		5.5	6	4		25.1
1892	AA108964	RT Alone		5.5	3	2		25.3
2189	AA113941	RT Alone		9.5	11	7.5		24.8
2190	AA113941	RT Alone		3.5	3	2		20
2192	AA113941	RT Alone		8.5	8	4		24.2
2201	AA113944	RT Alone		6	7	4		22.3
2202	AA113944	RT Alone		0				27.8
2203	AA113944	RT Alone		9	10	4.5		26.5
2204	AA113944	RT Alone		14	11	8		25.5
2342	AA117024	RT Alone		14	15.5	6		23.6
2343	AA117024	RT Alone		10	9	6		23.4
2344	AA117024	RT Alone		12	10	6.5		24
2345	AA117024	RT Alone		10	12	4		23.8
2346	AA117025	RT Alone		13	9.5	7		19.4
2347	AA117025	RT Alone		12	11.5	8		23.4
2348	AA117025	RT Alone		15.5	9	8		18.4
2349	AA117025	RT Alone		13	10	7		20.7
2350	AA117026	RT Alone		14	9.5	7		21.8
2351	AA117026	RT Alone		12.5	8	6.5		22.9
2352	AA117026	RT Alone		9	8	5		23.4
2353	AA117026	RT Alone		10	8	5		24.3

1863	AA108971	10^7 3616+RT		5	4	3		23.8
1864	AA108971	10^7 3616+RT		9	8	4		26.3
1865	AA108971	10^7 3616+RT	<10 d38	0				25.8
1866	AA108971	10^7 3616+RT	<10 d31	0				25.7
1883	AA108966	10^7 3616+RT	<10 d24	0				22.8
1884	AA108966	10^7 3616+RT	<10 d56	1	1	1		21.5
1886	AA108966	10^7 3616+RT	<10 d31	0				19.6
2074	AA108966	10^7 3616+RT		19	10	7		15.8
2032	AA111326	10^7 3616+RT		8.5	8.5	7		24.3
2033	AA111326	10^7 3616+RT		3.5	3.5	2.5		26.6
2035	AA111326	10^7 3616+RT		8	9	4		24.9
2072	AA111326	10^7 3616+RT	<10 d21	0				25.9
2036	AA111327	10^7 3616+RT		4.5	5	3		22.8
2037	AA111327	10^7 3616+RT	<10 d52	0				25.9
2039	AA111327	10^7 3616+RT		7	4.5	2.5		21.2
2073	AA111327	10^7 3616+RT		0				21.1
1871	AA108969	10^7 899-6+RT	<10 d66	0				25.8
1873	AA108969	10^7 899-6+RT		4	7	3.5		26.1
1874	AA108969	10^7 899-6+RT	<10 d52	0				28.9
1880	AA108967	10^7 899-6+RT	<10 d14	0				20.4
1881	AA108967	10^7 899-6+RT	<10 d42	9	10.5	6		28.8
1882	AA108967	10^7 899-6+RT	<10 d24	0				24.3
2044	AA111329	10^7 899-6+RT		8	9.5	6		25.8
2046	AA111329	10^7 899-6+RT	<10 d77	0				25.4
2048	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	4	2.5	1.5		26.2
2051	AA111330	10^7 899-6+RT	SCC	16	26	12		-

3616

2209	10	8.5	6	25.2
2210	9	7	5	25.2
2211	13	9.5	9.5	21.8
2212	8.5	9	6.5	22.6

6/15/95.

Greg's Mouse Log

Mouse #	Cage #	Group						weight
2207	AA113945	Control						
2354	AA117027	Control						
2356	AA117027	Control						
2357	AA117027	Control						
2359	AA117028	Control						
2360	AA117028	Control						
2361	AA117028	Control						
1710	AA105283	10^7 899-6	<10 d35	0				
2075	AA105283	10^7 899-6	<10 d42	0				
1713	AA105283	10^7 899-6	<10 d14	8				
2054	AA111331	10^7 899-6	<10 d49	0				
1723	AA104549	10^7 3616	<10 d28 face	0				
~~1859~~	AA108972	RT Alone						
1862	AA108972	RT Alone		5	6	4.5		
1892	AA108964	RT Alone		4.5	3	2.5		
2189	AA113941	RT Alone		10	13	6		
2190	AA113941	RT Alone		4	3	1.5		
2192	AA113941	RT Alone		8	7	4		
2201	AA113944	RT Alone		6	7	4		
2202	AA113944	RT Alone		0				
2203	AA113944	RT Alone		9	9	4		
2204	AA113944	RT Alone		13.5	11	9		
2342	AA117024	RT Alone		8	13.5	5.5		
2343	AA117024	RT Alone		10	9	6		
2344	AA117024	RT Alone		11.5	9	6.5		
2345	AA117024	RT Alone		13	14.5	9		
2346	AA117025	RT Alone		13	11	8		
2347	AA117025	RT Alone		10.5	10.5	7		
2348	AA117025	RT Alone		15	9.5	8.5		
2349	AA117025	RT Alone		10	14	7.5		
2350	AA117026	RT Alone		14	10.5	7		
2351	AA117026	RT Alone		10	8	6		
2352	AA117026	RT Alone		9.5	8.5	6		
2353	AA117026	RT Alone		12	9	~~6~~ 7		

3616
2209
2210
2211
2212

1863	AA108971	10^7 3616+RT		4.5	3	2.5		
~~1864~~	AA108971	10^7 3616+RT		8.5	6	3		
1865	AA108971	10^7 3616+RT	<10 d38	0				
~~1866~~	~~AA108971~~	~~10^7 3616+RT~~	~~<10 d31~~	0				
1883	AA108966	10^7 3616+RT	<10 d24	0				
1884	AA108966	10^7 3616+RT	<10 d56	+1.5	1.5	1.5		
1886	AA108966	10^7 3616+RT	<10 d31	0				
~~2074~~	AA108966	10^7 3616+RT	sac wt loss	+21.4	13.5	7		
2032	AA111326	10^7 3616+RT		+8	8	6		
2033	AA111326	10^7 3616+RT		3	2	1.5		
2035	AA111326	10^7 3616+RT		8.5	8.5	5		
2072	AA111326	10^7 3616+RT	<10 d21	0				
2036	AA111327	10^7 3616+RT		5	4.5	3		
2037	AA111327	10^7 3616+RT	<10 d52	0				
2039	AA111327	10^7 3616+RT		6	4.5	2.5		
~~2073~~	~~AA111327~~	~~10^7 3616+RT~~		0				
1871	AA108969	10^7 899-6+RT	<10 d66	6	4.5	3		
1873	AA108969	10^7 899-6+RT		4.5	5.5	3.5		
1874	AA108969	10^7 899-6+RT	<10 d52	0				
1880	AA108967	10^7 899-6+RT	<10 d14	0				
1881	AA108967	10^7 899-6+RT	<10 d42	10	10.5	6		
1882	AA108967	10^7 899-6+RT	<10 d24	0				
2044	AA111329	10^7 899-6+RT		9	8	9		
2046	AA111329	10^7 899-6+RT	<10 d77	0				
~~2048~~	AA111330	10^7 899-6+RT	<10 d38					
2050	AA111330	10^7 899-6+RT	<10 d31	0				
~~2051~~	AA111330	10^7 899-6+RT						

Greg's Mouse Log

Mouse #	Group					weight					weight
1710	10^7 899-6	0	–	–		26.7					
2075	10^7 899-6	0	0	0		26.7					
1713	10^7 899-6	0	–	–		23.0					
2054	10^7 899-6	6	–	–		25.1					
1723	10^7 3616	0	–	–	27.8	18.2					
1862	RT Alone	11	8	7		26.1					
1892	RT Alone	3	3	2		26.1					
2189	RT Alone	6	7	5	25.0	23.2					
2190	RT Alone	0				25.3					
2192	RT Alone	4	4	1.5		25.0					
2201	RT Alone	3	3	1.5		27.3					
2202	RT Alone	0	–	–		24.6					
2203	RT Alone	5	4	1.5		23.6					
2204	RT Alone	Sacrificed									
2342	RT Alone	17	17	12		25.0					
2343	RT Alone	12	11	8		24.3					
2344	RT Alone	12	9	8		24.7					
2345	RT Alone	7	3	1.5		25.5					
2346	RT Alone	12	10	7		23.4					
2347	RT Alone	18	15	11		26.3					
2348	RT Alone	16	13	9		20.4					
2349	RT Alone	14	10	8		21.2					
2350	RT Alone	13	10	8		25.4					
2351	RT Alone	8	7	6		25.1					
2352	RT Alone	9	8	5		24.6					
2353	RT Alone	11	7	4		25.5					
1863	10^7 3616+RT	3	3	1		24.7					
1864	10^7 3616+RT	0	–	–		27.7					
1865	10^7 3616+RT	6	–	–		27.3					
1866	10^7 3616+RT	0	–	–		26.4					
1883	10^7 3616+RT	0	–	–		24.5					
1884	10^7 3616+RT										
1886	10^7 3616+RT	0	–	–		25.4					
2032	10^7 3616+RT	12	9	8		25.7					
2033	10^7 3616+RT	2	2	1		26.6					
2035	10^7 3616+RT	3	2	1		24.7					
2072	10^7 3616+RT	0	–	–		23.9					
2036	10^7 3616+RT	3	3	2		21.4					
2037	10^7 3616+RT	1	1	1		26.4					
2039	10^7 3616+RT	0	–	–		19.0					
2073	10^7 3616+RT	0	–	–		21.3					
1871	10^7 899-6+RT	0	–	–		26.4					
1873	10^7 899-6+RT	0	–	–		27.2					
1874	10^7 899-6+RT	0	–	–		25.6					
1880	10^7 899-6+RT	0	–	–		19.8					
1881	10^7 899-6+RT	15	13	11		27.2					
1882	10^7 899-6+RT	0	–	–		24.0					
2044	10^7 899-6+RT	11	10	7	26.4 →						
2046	10^7 899-6+RT	0	–	–	27.0 →						
2050	10^7 899-6+RT	1	1	1	27.0 →						

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Greg's Mouse Log

Mouse #	Group					weight					weight
1710	10^7 899-6	0				25.8					
2075	10^7 899-6	0				25.2					
1713	10^7 899-6	0				22.7					
2054	10^7 899-6	0				24.3					
1723	10^7 3616	0				26.3					
1862	RT Alone	8	7	4		23.2					
1892	RT Alone	4	4	2		22.9					
2189	RT Alone	14	11	10		25.3					
2190	RT Alone	0				22.0					
2192	RT Alone	6	6	6		25.0					
2201	RT Alone	5	5	4		28.0					
2202	RT Alone	0				27.4					
2203	RT Alone	9	9	6		23.3					
2204	RT Alone	18	17	10		27.6					
2342	RT Alone	13	12	6		25.0					
2343	RT Alone	12	7	7		26.3					
2344	RT Alone	19	15	9		25.3					
2345	RT Alone	10	8	5		25.7					
2346	RT Alone	11	11	8		22.8					
2347	RT Alone	15	14	9		26.0					
2348	RT Alone	16	9	7		18.3					
2349	RT Alone	16	16.5	6		22.2					
2350	RT Alone	15	11	6		25.5					
2351	RT Alone	9	8	5		24.2					
2352	RT Alone	9	9	4		24.9					
2353	RT Alone	11	12	6		23.3					
1863	10^7 3616+RT	4	4	2		25.3					
1864	10^7 3616+RT	9	8	1.5		25.6					
1865	10^7 3616+RT	0				27.4					
1866	10^7 3616+RT	0				26.7					
1883	10^7 3616+RT	0				23.4					
1884	10^7 3616+RT	0				19.5					
1886	10^7 3616+RT	0				22.6					
2032	10^7 3616+RT	0				26.0					
2033	10^7 3616+RT	4	3	1.5		26.5					
2035	10^7 3616+RT	7	6	4		24.7					
2072	10^7 3616+RT	9	7	5		26.8					
2036	10^7 3616+RT	6	5	1.5		23.9					
2037	10^7 3616+RT	0				27.3					
2039	10^7 3616+RT	5	5	1.5		19.9					
2073	10^7 3616+RT	0				22.5					
1871	10^7 899-6+RT	0				25.9					
1873	10^7 899-6+RT	7	6	1.5		26.1					
1874	10^7 899-6+RT	0				27.0					
1880	10^7 899-6+RT	0				19.8					
1881	10^7 899-6+RT	15	12	7		32.1					
1882	10^7 899-6+RT	0				23.9					
2044	10^7 899-6+RT	14	14	7		28.2					
2046	10^7 899-6+RT	0				26.3					
2050	10^7 899-6+RT	0				26.4					

Data Sheet

		7/13/95	7/17/95			
Greg's Mouse	Data					
Mouse#	Group	Measurements	Weight			
2189	RT Alone	6x6x3	7x6x3	26.7		
2190	RT Alone	0x--	0	23.4		
2192	RT Alone	2x2x1	1x1x1	25.9		
2201	RT Alone	2x2x1.5	3x3x1.5	29.2		
2202	RT Alone	0x--	0	29.9		
2203	RT Alone	4x4x3	4x3x3	23.6		
~~2204~~	~~RT Alone~~		SAC			
2342	RT Alone	11x10x9	11x9x3	21.3		
2343	RT Alone	12x10x9	~~[illegible]~~	24.4	11x10x7	
2344	RT Alone	13x12x13	19x13x12	24.0		
2345	RT Alone	7x3x1.5	6x3x1.5	24.1		
2346	RT Alone	12x10x7	9x7x5	23.4		
2347	RT Alone	~~13x12x7~~ 13x15x12	17x15x11	26.3		
2348	RT Alone	13x12x8	16x14x9	21.4		
sick 2349	RT Alone	14x10x10	14x10x8	17.4		
2350	RT Alone	12x9x8	12x9x6	26.1		
2351	RT Alone	8x6x5	9x7x4	21.9		
2352	RT Alone	9x8x4	9x9x3	26.0		
2353	RT Alone	11x6x5	9x6x4	25.1		
2687	RT Alone	12x10x8	11x9x7	21.0		
2688	RT Alone	13x11x9	~~[illegible]~~	~~[illegible]~~	12x11x8	21.8
2689	RT Alone	13x8x8	14x8x7	23.9		
2690	RT Alone	10x7x5	9x7x6	24.5		
2354	Control					
2355	Control					
2356	Control					
2357	Control					
2358	Control					
2359	Control					
2360	Control					
2361	Control					
* 2667	Control	19x16x15		deceased 7/17/95		
2668	Control	16x15x12	16x14x13	18.5		
2669	Control	16x15x19	18x16x13	23.0		
2670	Control	22x15x12	22x15x10			

Data Sheet

		7/18/95	7/17/95	
2679	10^7 3636RT	6x6x3	5x5x2	
2680	10^7 3616RT	8x6x3	7x4x2	
2681	10^7 3636RT	12x10x7	11x9x4	
2682	10^7 3616RT	13x8x8	13x9x6	
2691	10^7 3636RT	~~13x10x9~~ 7/17/[illegible]	13x11x9	21.5
2692	10^7 3616RT	11x8x8	14x10x8	23.2
2693	10^7 3636RT	13x10x8	[illegible]x6	22.6
2694	10^7 3616RT	12x9x9	11x9x6	25.7
2683	10^7 3616RT	8x7x6	8x7x4	21.4
2684	10^7 3636RT	7x7x5	6x5x3	17.1
2685	10^7 3616RT	8x7x5	6x4x3	23.1
2686	10^7 3616RT	10x7x7	8x6x6	A-3
2675	10^7 8996RT	10x9x7	[illegible]	10x7x5
2676	10^7 8996RT	12x12x9	13x9x8	
2677	10^7 8996RT	14x9x8	12x9x4	
2678	10^7 8996RT	14x9x9	13x10x6	
2671	10^7 8996RT	8x6x4	6x5x4	
2672	10^7 8996RT	9x7x6	8x6x5	
2673	10^7 8996RT	10x10x9	13x11x6	
2674	10^7 8996RT	10x8x4	9x8x1.5	
2695	10^7 8996RT		10x8x8	
INJ 2696	10^7 8996RT		17x12x9	
2697	10^7 8996RT		10x11x7	
2698	10^7 8996RT		11x11x6	
2663	899-6	13x10x9	16x12x8	
2664	899-6	23x19x15	27x25x15	→ SAC 7/17
2665	899-6	~~SAC 7/6~~		
2666	899-6	14x9x8	19x15x10	
2703	899-6	9x7x5	[illegible]	11x7x4
2704	899-6	11x9x6	11x11x3	
2705	899-6	14x12x10	18x14x7	
2706	899-6	16x11x9	17x11x9	
	899-6			
	899-6			
	899-6			
	899-6			

Data Sheet

			7/13/95	7/17/95	
	2659	3616	14x14x14	15x15x18	
	2660	3616	22x18x13	28x19x11	⇒ SAC 7/17
	2661	3616	19x15x11	18x16x7	
	2662	3616	23x15x13	27x17x12	⇒ SAC 7/17
AA12008	2707	3616	13x10x7		weighed 7/18/95
	2708	3616	12x9x6		
	2709	3616	11x9x7		
	2710	3616	13x11x8		
AA 12081	2719	3616	9x8x6		7/18/95
	2720	3616	7x8x7		
	2721	3616	9x8x7		
	2722	3616	10x8x6		

Data Sheet

Greg's Mouse	Data	7/20/95	7/20/95
Mouse#	Group	Measurements	Weight
2189	RT Alone	9x5x1.5	25.8
2190	RT Alone	0	23.9
2192	RT Alone	3x3x2	25.9
2201	RT Alone	5x2x1.5	28.1
2202	RT Alone	0	29.3
2203	RT Alone	4x3x1.5	24.2
2204 SAC	RT Alone		
2342	RT Alone	11x9x4	21.9
2343	RT Alone	12x12x6	24.1
2344	RT Alone	22x14x10	24.0
2345	RT Alone	6x3x1.5	24.3
2346	RT Alone	9x8x5	23.5
2347	RT Alone	16x16x10	25.2
2348	RT Alone	15x15x10	21.0
~~2349~~	~~RT Alone~~	died 7/20/95	
2350	RT Alone	8x8x3	24.3
2351	RT Alone	12x9x5	27.1
2352	RT Alone	8x8x3	25.7
2353	RT Alone	9x6x4	25.3
2687	RT Alone	12x9x6	22.0
2688	RT Alone	12x10x6	23.5
2689	RT Alone	12x8x5	24.7
2690	RT Alone	9x7x6	25.1
2354	Control		
2355	Control		
2356	Control		
2357	Control		
2358	Control		
2359	Control		
2360	Control		
2361	Control		
2667 SAC	Control		
2668	Control		
2669	Control	19x14x14	22.7
2670	Control	21x13x8	17.4

died 7/20/95

Data Sheet

		7/20/95	7/20/95
2679	10^7 3636RT	5x4x1.5	22.4
2680	10^7 3616RT	7x4x1.5	13.7
2681	10^7 3636RT	11x7x5	24.2
2682	10^7 3616RT	13x8x8	20.6
2691	10^7 3636RT	7x6x4	23.5
2692	10^7 3616RT	9x8x6	21.1
2693	10^7 3636RT	12x10x3	22.8
2694	10^7 3616RT	13x9x7	24.4
2683	10^7 3616RT	7x6x4	28.2
2684	10^7 3636RT	0	19.0
2685	10^7 3616RT	6x5x1.5	23.4
2686	10^7 3616RT	10x8x4	18.9
2675	10^7 8996RT	12x8x5	25.2
2676	10^7 8996RT	13x12x7	17.3
2677	10^7 8996RT	12x9x5	19.1
2678	10^7 8996RT	12x10x6	25.8
2671	10^7 8996RT	7x7x4	22.0
2672	10^7 8996RT	8x6x4	25.1
2673	10^7 8996RT	14x11x6	20.1
2674	10^7 8996RT	11x8x4	14.4
2695	10^7 8996RT	10x9x6	22.7
2696	10^7 8996RT	17x13x9	19.7
2697	10^7 8996RT	10x10x6	24.9
2698	10^7 8996RT	12x10x8	17.1
2663	899-6	16x15x7	22.4
2664 SAC	899-6		
2665	899-6	21x17x13	24.3
SAC ~~2666~~	899-6		
2703	899-6	13x8x5	25.2
2704	899-6	12x11x4	27.3
2705	899-6	21x15x9	26.3
2706	899-6	19x13x6	21.0
	899-6		
	899-6		
	899-6		
	899-6		

In Vitro U-251 10/6/95

Need 0 PFU < 0 d / 9 d < 6 d

0.1 PFU < 0 d / 9 d < 6 d

10 PFU < 0 d / 9 d < 6 d

Need 9 plates plus 1 plate to count
set up 12 plates

1) Tryp
2) Count cells $148 \times 12 \times 10^4 = 163 \times 10^5 - 16 \times 10^6$ resuspend in 12 ml
3) Add 10 ml media/plate + 1/2 ml cell soln.

10/10/95

Plates 80-90% confluent
Count cells in spare plate 78-80

$80 \times 12 \times 10^4 = 960 \times 10^4 = 9.6 \times 10^6 = 10 \times 10^6$

will infect c̄ 0.1 PFU and 1 PFU

0.1 PFU (10×10^5) PFU $10 \times 10^5 PFU \left(\frac{1 ml}{2 \times 10^7 PFU}\right) = \frac{5}{100} ml \cdot \frac{1000 \lambda}{1 ml} = 50 \lambda$

∴ 150 λ in 9 ml

1 PFU (10×10^6) PFU $10 \times 10^6 PFU \left(\frac{1 ml}{9 \times 10^8 PFU}\right) = \frac{1.1}{10^2} \frac{10^3}{1 ml} = 11 \lambda$/plate

4.4 λ in 12 ml

Infect at 9:45 am

10/11/95

4) Harvest 0 d at 9:40 am
5) Harvest plates 1, 2 / 4, 5 / 7, 8 at 6:00
6) Plates 3, 6, 9 24h (90%) 20-30% (somewhat growing)

In Vitro 1-87 Cytotoxicity (cont.) 10/9/95

Plates infected c̄ 0, 0.1, 1, 10 PFU at 11[30] am
2° post inf., add 2nd serum(-) media

LT Plates 10/10/95
Irradiate at 8[00] am (~20°)
harvest at 2[00] pm (6° post LT) and 8[00] am 10/11/95
(12 ... 4, 5 ... 8
rounding ... similar ... cell rounding
(no cell loss)

10/11/95
Harvest at 24° 8[30] am

3 ... 6 ... 9
same ... lots of cell rounding
+ death

D-54 (GBM) in nude mice cytoxan 10/10/95

Receive 3 mice from MIT ē D54 in (B) hind leg

	ear tag	(L) mm³		(R) mm³	
5008	Ø	14x9x[illegible]	3816	10x[illegible]	3816
5009	L	16x11x8	3816	15x10x[illegible]	3816
5010	R	12x[illegible]x5	3816	13x[illegible]x9	3816

Inject mice ē total 3816 (2x10[illegible]) per kg

Irradiate Mice ē 10/11/95

Irradiate 3 mice ē 20 Gy to (R) hind limbs
* 5009 partially irradiate 2° to tumor size

10/12/95

Sac Mice

		L mm³	R	L (g)	R
Day 1	5008	14x10x9	11x10x9	5.13	5.15
				5.56	5.62
				0.43	.47g
Day 2	5009	17x15x12	18x15x14	5.17	~~5.1~~ 5.1
				6.73	7.06
				1.56	1.98
	5010	12x10x8	11x9x6	5.18	5.03
				5.98	5.39
				0.80	.36

In Vitro (j-77) 10/10/95

Repeat of expt on 10/6/95 (that expt had ↑ loss of cells even those of ctrl plates)

also will do protein [illegible] infection

LT 6hrs/6hrs post infection

0 PFU ⟨ 0 Gy 1; 9 Gy ⟨ 60 2; 240 3

0.1 PFU ⟨ 0 Gy 4; 9 Gy ⟨ 60 5; 240 6

1.0 PFU ⟨ 0 Gy 7; 9 Gy ⟨ 60 8; 240 9

Plate ~ 2×10^6 cells / 100mm plate
in 10ml media

10/12/95

cells ~ 80% confluent
$70 \times 10 \times 10^4 = 7 \times 10^6$ cells/plate

1PFU 7×10^6 PFU $\frac{1\,ml}{9 \times 10^9\,PFU}$ ~ 1 λ

∴ 4 λ in 12 ml (2%)
3 ml/plate

0.1PFU
1ml 1PFU + 9ml media (2%)
~10[15] 3 ml/plate

infect 2°, then add 2 ml 10% media
LT 6° post infection ~ 4[15]

Harvest at 10[15] 1,2 4,5 7,8
90% 70% 60% 30%

10/13/95

4[15] 3 6 9
90% 50% 25%

Data Sheet

7/20/95

2659	3616	20 x 20 x 14	24.8
2660 SAC	3616		
2661	3616	21 x 16 x 12	23.2
2662 SAC	3616		
2707	3616		
2708	3516		
2709	3616		
2710	3616		
2719	3616		
2720	3616		
2721	3616		
2722	3616		

Data Sheet

Greg's Mouse	Data	7/24/95	7/27/95	
Mouse#	Group	Measurements	Weight	
2189	RT Alone	8×8×1.5	5×5×1.5	28.3
2190	RT Alone	∅	∅	25.9
2192	RT Alone	∅	∅	23.3
2201	RT Alone	5×3×1.5	5×3×1.5	24.8
2202	RT Alone	∅	∅	24.1
2203	RT Alone	5×4×1.5	6×4×1.5	23.3
~~2204~~ SAC	~~RT Alone~~			
2342	RT Alone	20×17×12	22×17×12	24.6
2343	RT Alone	12×10×5	12×10×6	24.7
2344	RT Alone	∅	∅	23.1
2345	RT Alone	∅	∅	24.7
2346	RT Alone	11×7×3	8×7×3	25.1
2347	RT Alone	15×15×10	17×15×10	26.0
2348	RT Alone	15×15×9	14×11×9	20.8
SAC ~~2349~~	~~RT Alone~~			
2350	RT Alone	12×9×6	10×9×5	27.2
2351	RT Alone	10×8×4	10×7×3	25.5
2352	RT Alone	9×9×1.5	9×9×1.5	26.2
2353	RT Alone	10×7×2	9×6×4	25.7
2687	RT Alone	11×9×6	11×10×4	28.6
2688	RT Alone	12×9×5	12×9×4	25.3
2689	RT Alone	11×9×8	12×9×4	24.3
2690	RT Alone	10×8×4	9×7×4	27.3
~~2354~~	~~Control~~			
~~2355~~	~~Control~~			
~~2356~~	~~Control~~			
~~2357~~	~~Control~~			
~~2358~~	~~Control~~			
~~2359~~	~~Control~~			
~~2360~~	~~Control~~			
~~2361~~	~~Control~~			
~~2667~~ SAC	~~Control~~			
~~2668~~	~~Control~~			
~~2669~~	~~Control~~			
~~2670~~	~~Control~~			

Data Sheet

7/14/95		7/14/95	7/28/95	
~~SAC 2659~~	~~3616~~	~~21x21x15~~		
~~2660 SAC~~	~~3616~~			
~~SAC 2661~~	~~3616~~	~~25x19x13~~		
~~2662 SAC~~	~~3616~~			
2707	3616	11x9x7	11x9x4	23.8
2708	3616	12x10x6	12x10x5	22.8
2709	3616	14x12x7	14x13x10	25.0
2710	3616	13x11x7	14x11x8	25.0
2719	3616	7x7x4	11x5x15	22.6
2720	3616	11x9x5	8x6x3	25.1
2721	3616	7x6x4	13x9x6	23.3
2722	3616	9x8x4	9x6x4	22.7

Data Sheet

Greg's Mouse	Data	7/31/15	8/3/15	
Mouse#	Group	Measurements	Weight	
2189	RT Alone	0	0	24.5
2190	RT Alone	0	0	22.4
2192	RT Alone	0	0	25.0
2201	RT Alone	0	0	27.6
2202	RT Alone	5x3x1.5	0	28.4
2203	RT Alone	4x4x1.5	4x4x1.5	22.9
SAC ~~2204~~	~~RT Alone~~			
2342	RT Alone	23x19x7	23x19x9	21.0
2343	RT Alone	11x10x6	12x11x5	23.5
2344	RT Alone	0	0	23.0
2345	RT Alone	0	0	23.5
2346	RT Alone	0	0	~~[illegible]~~ 21.3
2347	RT Alone	19x17x11	19x17x11	26.8
2348	RT Alone	14x12x10	14x12x10	24.6
SAC ~~2349~~	~~RT Alone~~			
2350	RT Alone	9x8x3	9x8x3	24.4
2351	RT Alone	8x7x3	9x6x2	25.4
2352	RT Alone	10x9x3	8x8x1.5	26.6
2353	RT Alone	9x5x1.5	8x5x2	25.5
2687	RT Alone	10x7x4	11x7x5	24.0
2688	RT Alone	9x9x4	11x11x4	25.6
2689	RT Alone	14x13x6	15x13x9	24.1
2690	RT Alone	8x7x2	9x7x3	26.9
2679	10^7 3636RT	0	0	22.7
died 8/1/15 ~~2680~~	~~10^7 3616RT~~	0		
2681	10^7 3636RT	9x7x3	6x6x2	15.6
2682	10^7 3616RT	12x6x4	12x6x5	21.5
2691	10^7 3636RT	4x4x1.5	3x3x1.5	23.2
2692	10^7 3616RT	10x6x3	8x6x2	27.7
2693	10^7 3636RT	8x7x3	8x7x1.5	23.4
2694	10^7 3616RT	11x9x4	11x9x4	25.9
2683	10^7 3616RT	6x4x1.5	3x3x1.5	23.3
2684	10^7 3636RT	0	0	21.8
2685	10^7 3616RT	0	0	25.1
2686	10^7 3616RT	5x5x2	4x4x1.5	20.8
2675	10^7 8996RT	7x5x1.5	4x5x2	27.1
2676	10^7 8996RT	10x9x4.5	8x8x4	19.1
2677	10^7 8996RT	7x6x2	5x4x1.5	22.6
2678	10^7 8996RT	8x8x3	8x8x3	28.0

Data Sheet

7/31/95

	2671	10^7 8996RT	2x2x1.5	2x2x1.5	22.2
	2672	10^7 8996RT	5x3x1.5	2x2x1.5	25.5
	2673	10^7 8996RT	9x7x3	9x7x2	18.6
	2674	10^7 8996RT	6x4x1.5	4x3x1.5	22.9
	2695	10^7 8996RT	7x6x3	6x5x2	21.7
	2696	10^7 8996RT	[illegible]	16x11x7	20.9
	2697	10^7 8996RT	9x7x6	9x7x3	27.5
	2698	10^7 8996RT	10x9x5	11x9x7	16.8
	2663	899-6	16x11x5	17x13x6	21.2
	~~SAC 2664~~	~~899-6~~			
	~~SAC 2665~~	~~899-6~~			
	~~SAC 2666~~	~~899-6~~			
	2703	899-6	21x16x7	21x19x7	27.3
	2704	899-6	22x16x6	23x15x5	21.5
died 8/1/95	2705	899-6	14x15x8		
	2706	899-6	18x18x6	17x15x4	19.7
injected 8/1/95	2711	899-6	15x13x8		
	2712	899-6	13x10x8		
	2713	899-6	11x11x10		
	2714	899-6	14x10x10		
	2707	3616	13x10x4	15x13x6	27.2
	2708	3616	11x10x6	12x11x6	23.4
	2709	3616	17x15x9	20x16x9	26.9
	2710	3616	17x13x7	17x15x10	25.7
	2719	3616	5x3x1.5	2x2x1.5	23.9
	2720	3616	5x4x1.5	2x2x1.5	26.3
	2721	3616	11x9x3	13x11x6	24.9
	2722	3616	7x6x2	6x6x4	24.5

Data Sheet

Greg's Mouse	Data	8/7/95	8/10/95	
Mouse#	Group	Measurements	Weight	
2189	RT Alone	1.5x1.5x1	1.0x1.0x1	25.0
2190	RT Alone	0	0	24.3
2192	RT Alone	0	0	26.3
2201	RT Alone	0	0	29.7
2202	RT Alone	0	0	30.7
2203	RT Alone	1.5x1.5	1.5x1.5x1.5	24.5
~~SAC 2204~~	~~RT Alone~~			
2342	RT Alone	22x19x9	20x15x8	23.5
2343	RT Alone	12x10x4	13x12x6	22.7
2344	RT Alone	0	0	21.5
2345	RT Alone	0	0	24.7
2346	RT Alone	0	0	24.5
died 8/7/95 2347	RT Alone	20x18x9	[illegible]	
2348	RT Alone	15x11x6	15x12x6	20.1
~~SAC 2349~~	~~RT Alone~~			
2350	RT Alone	8x7x3	8x6x4	27.5
2351	RT Alone	10x7x3	9x7x4	26.0
2352	RT Alone	10x9x2	8x8x2	28.7
2353	RT Alone	8x5x2	8x5x3	26.7
2687	RT Alone	11x8x5	9x8x5	25.1
2688	RT Alone	10x9x4	10x9x5	28.3
2689	RT Alone	12x12x9	15x12x12	24.3
2690	RT Alone	9x6x3	7x6x3	28.5
2679	10^7 3636RT	0	0	24.9
~~SAC 2680~~	~~10^7 3616RT~~			
2681	10^7 3636RT	12x8x5	12x9x5	23.3
8/4/95 ~~2682~~	~~10^7 3616RT~~			
2691	10^7 3636RT	7x5x1.5	0	23.1
2692	10^7 3616RT	~~[illegible]~~ 0	6x6x1.5	24.0
2693	10^7 3636RT	7x7x1.5	8x6x1.5	24.6
2694	10^7 3616RT	10x7x4	10x7x3	26.6
2683	10^7 3616RT	0	0	24.6
2684	10^7 3636RT	0	0	22.0
2685	10^7 3616RT	0	0	.4
2686	10^7 3616RT	4x3x1.5	1.0x1.0x1	.2
2675	10^7 8996RT	4x4x1.5 ~~[illegible]~~	4x4x1.5	2.3
2676	10^7 8996RT	8x8x4	9x7x4	20.3
2677	10^7 8996RT	1.5x1.5x1.5	4x4x1.5	24.7
2678	10^7 8996RT	9x8x3	8x6x3	29.5

HISTO-PATHOLOGICAL

	3d	6d	9d
Control			
RT alone			
RT + 3616			
3616 Alone			

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Data Sheet

Greg's Mouse	Data	8/14/95	8/17/95	
Mouse#	Group	Measurements	Weight	
2342	RT Alone	20x14x8	20x14x8	26.2
2343	RT Alone	14x10x7	12x11x7	23.1
2344	RT Alone	Ø	Ø	20.0
2345	RT Alone	Ø	Ø	25.2
2346	RT Alone	Ø	Ø	25.7
SAC 2347	RT Alone	[illegible]	[illegible]	
2348	RT Alone	14x14x6	16x14x6	21.6
SAC 2349	RT Alone	[illegible]	[illegible]	
2350	RT Alone	10x8x4	7x6x3	28.1
2351	RT Alone	9x6x2	8x7x3	26.2
2352	RT Alone	11x7x2	9x7x3	28.7
2353	RT Alone	7x6x2	7x5x2	26.1
2687	RT Alone	9x7x4	10x7x4	24.7
2688	RT Alone	10x7x4	10x10x4	29.0
2689	RT Alone	13x11x3	13x12x5	23.6
2690	RT Alone	8x6x3	8x6x3	27.9
2679	10^7 3636RT	Ø	Ø	25.2
SAC 2680	10^7 3616RT	[illegible]	[illegible]	
2681	10^7 3636RT	12x10x6	12x10x9	22.6
SAC 2682	10^7 3616RT	[illegible]	[illegible]	
2691	10^7 3636RT	Ø	Ø	23.3
2692	10^7 3616RT	6x6x1.5	6x5x1.5	30.3
2693	10^7 3636RT	6x5x1.5	5x4x1.5	25.2
2694	10^7 3616RT	8x6x2	8x6x2	26.8
2683	10^7 3616RT	Ø	Ø	24.6
2684	10^7 3636RT	Ø	Ø	22.4
2685	10^7 3616RT	Ø	Ø	26.4
2686	10^7 3616RT	1.0x1.0x1.0	Ø	28.4
2675	10^7 8996RT	4x4x1.5	4x4x1.5	27.8
2676	10^7 8996RT	10x8x4	8x9x6	21.6
2677	10^7 8996RT	4x3x1.5	6x4x1.5	23
2678	10^7 8996RT	8x6x3	7x6x2	28.6
2671	10^7 8996RT	Ø	Ø	24.4
2672	10^7 8996RT	Ø	Ø	27.8
2673	10^7 8996RT	5x5x1.5	4x4x1.5	26.0
2674	10^7 8996RT	Ø	Ø	24.5
2695	10^7 8996RT	3x3x1.0	2x2x1.5	24.5
2696	10^7 8996RT	11x10x6	11x10x4	23.0
2697	10^7 8996RT	7x5x2	6x4x2	26.0

Data Sheet

8/21/95 8/24/95

Greg's Mouse	Data			
Mouse#	Group	Measurements	Weight	
2346	RT Alone	φ	φ ♀ 25.3	φ
SAC 2347	RT Alone	—	—	
2348	RT Alone	16x14x7	17x14x8 21.7	
SAC 2349	RT Alone	—	—	
2350	RT Alone	9x7x3	8x6x3 22.3	
2351	RT Alone	8x6x3	8x7x3	25.8
2352	RT Alone	7x7x2	10x8x3	28.1
2353	RT Alone	7x5x3	7x5x2	26.3
2687	RT Alone	9x8x5	10x8x3	25.8
2688	RT Alone	~~8~~ 11x10x4	11x9x4	28.9
2689	RT Alone	15x12x4	16x13x5	27.8
2690	RT Alone	8x6x3	7x5x3	28.2
2679	10^7 3636RT	φ	φ	25.3
SAC 2680	10^7 3616RT	—	—	
2681	10^7 3636RT	13x12x9	13x12x8	21.0
SAC 2682	10^7 3616RT	—	—	
2691	10^7 3636RT	6x5x1.5	6x4x1.5	29.2
2692	10^7 3616RT	φ	φ	22.9
2693	10^7 3636RT	4x4x1.5	4x3x1.5	24.7
2694	10^7 3616RT	6x5x2	6x6x2	27.0
2683	10^7 3616RT	φ	φ	24.0
2684	10^7 3636RT	φ	φ	23.3
2685	10^7 3616RT	φ	φ	26.2
2686	10^7 3616RT	1x1x1	φ	22.1
2675	10^7 8996RT	6x6x2	5x5x2	27.5
2676	10^7 8996RT	9x9x4	10x9x5	21.4
2677	10^7 8996RT	5x3x1.5	5x3x1.5	24.2
2678	10^7 8996RT	6x5x2	6x5x1.5	29.1
2671	10^7 8996RT	φ	φ	23.8
2672	10^7 8996RT	φ	φ	27.8
2673	10^7 8996RT	4x2x1.5	4x1.5x1.5	26.2
2674	10^7 8996RT	φ	φ	24.5
2695	10^7 8996RT	1x1x1	1x1x1	28.5
2696	10^7 8996RT	11x10x6	10x9x5	23.0
2697	10^7 8996RT	7x5x2	8x5x3	25.3
2698	10^7 8996RT	11x8x5	12x10x4	17.1
dead 2714	899-6	13x16x13	—	
8/22/95				

Data Sheet

2719	3616	Φ	Φ	23.9
2720	3616	6x3x1.5	8x4x2	27.2
SAC 2721	3616	~~15x15x6~~	—	
2722	3616	15x15x6	15x15x6	24.9
2715	HSV/ GAN	18x15x11	~~dead sac~~	dead 8/23
2716	HSV/ GAN	21x18x10	23x18x15	21x17x11.0 21.8
2717	HSV/ GAN	14x11x8	16x13x7	25.9
2718	HSV/ GAN	22x18x10	23x18x15	25.6
2731	HSV/RT/GAN	6x6x1.5	6x6x3	23.4
2733	HSV/RT/GAN	13x13x4	12x11x3	23.6

Data Sheet

8/28/95

Greg's Mouse	Data		
Mouse#	Group	Measurements	Weight
2346	RT Alone	Ø	
SAC 2347	RT Alone		
2348	RT Alone	14x14x7	
SAC 2349	RT Alone		
2350	RT Alone	7x6x3	
2351	RT Alone	1x7x3	
2352	RT Alone	8x3x5	
2353	RT Alone	7x4x3	
2687	RT Alone	10x8x4	
2688	RT Alone	9x6x4	
2689	RT Alone	18x15x8	
2690	RT Alone	6x6x2	
2679	10^7 3636RT	Ø	
SAC 2680	10^7 3616RT		
2681	10^7 3636RT	13x11x7	
SAC 2682	10^7 3616RT		
2691	10^7 3636RT	Ø	
2692	10^7 3616RT	4x3x1.5	
2693	10^7 3636RT	1x1x0.5	
2694	10^7 3616RT	3x3x1.5	
2683	10^7 3616RT	Ø	
2684	10^7 3636RT	Ø	
2685	10^7 3616RT	Ø	
2686	10^7 3616RT	Ø	
2675	10^7 8996RT	4x3x2	
2676	10^7 8996RT	8x8x3	
2677	10^7 8996RT	4x3x1.5	
2678	10^7 8996RT	1x1x0.5	
2671	10^7 8996RT	Ø	
2672	10^7 8996RT	Ø	
2673	10^7 8996RT	1x1x0.5	
2674	10^7 8996RT	Ø	
2695	10^7 8996RT	Ø	
2696	10^7 8996RT	9x8x3	
2697	10^7 8996RT	6x6x3	
2698	10^7 8996RT	12x10x5	
SAC 2714	899-6		
8/29/95			

Data Sheet

8/28/95

2719	3616	0	
2720	3616	6x6x3	
SAG 2721	3616	[illegible]	[illegible]
2722	3616	16x15x5	
8/24/95 SAC ~~[illegible] HSV/ GAN~~			
2716	HSV/ GAN	18x16x8	
2717	HSV/ GAN	18x13x7	
SAC 8/28/95 2718	HSV/ GAN		
2735	rejected 8/28/95	14x[illegible]	
2731	HSV/RT/GAN	5x4x3	
2735	HSV/RT/GAN	10x10x3	

L 2732 rejected 8/28/95 9x9x6

Greg's Mouse	Data	8/31/95		9/4/95
Mouse#	Group	Measurements	Weight	
2687	RT Alone	11x9x3	25.5	9x7x3
2688	RT Alone	8x8x3	29.3	7x6x3
2689	RT Alone	19x17x8	27.8	20x18x6
2690	RT Alone	8x6x3	28.5	9x5x2
2679	10^7 3636RT	0	15.6	ϕ
SAC 2680	10^7 3616RT			—
2681	10^7 3636RT	13x13x7	20.7	13x13x8
SAC 2682	10^7 3616RT			—
2691	10^7 3636RT	0	23.5	ϕ
2692	10^7 3616RT	0	30.2	ϕ
2693	10^7 3636RT	0	25.1	ϕ
2694	10^7 3616RT	9x4x2	25.8	9x7x2
2683	10^7 3616RT	0	25.5	ϕ
2684	10^7 3636RT	0	23.7	ϕ
2685	10^7 3616RT	0	26.7	ϕ
2686	10^7 3616RT	0	21.8	ϕ
2675	10^7 8996RT	5x5x3	29.5	4x4x3
2676	10^7 8996RT	8x6x3	23.7	8x6x4
2677	10^7 8996RT	5x3x1.5	24.4	4x2x1.5
2678	10^7 8996RT	[illegible]x[illegible]x0.5	30.4	1x1x0.5
2671	10^7 8996RT	0	24.8	ϕ
2672	10^7 8996RT	0	21.2	ϕ
2673	10^7 8996RT	1x1x0.5	22.3	ϕ
2674	10^7 8996RT	0	25.6	ϕ
2695	10^7 8996RT	1x1x1	24.7	4x4x1
2696	10^7 8996RT	8x8x3	23.7	9x5x2
2697	10^7 8996RT	11x9x5	26.2	13x9x6
2698	10^7 8996RT	13x11x6	20.0	13x13x5
2719	3616	0	24.3	ϕ
2720	3616	7x7x3	28.4	10x9x6
SAC 2721	3616			
2722	3616	19x15x6	26.6	20x18x8
SAC 2715	HSV/ GAN			
2716	HSV/ GAN	20x16x10	21.9	20x16x8
2717	HSV/ GAN	24x18x10	26.7	24x20x13
SAC 2718	HSV/ GAN			

Data Sheet

Greg's Mouse	Data	9/7/95		9/10/95
Mouse#	Group	Measurements	Weight	
2687	RT Alone	9x6x3	26.1	7x5x2
2688	RT Alone	7x5x2	28.8	6x6x4
2689	RT Alone	19x16x6	24.5	18x14x6
2690	RT Alone	9x6x4	29.7	6x5x2
2679	10^7 3636RT	0	25.8	0
SAC 2680	10^7 3616RT			
2681	10^7 3636RT	15x13x8	20.7	15x14x8
SAC 2682	10^7 3616RT			
2691	10^7 3636RT	0	24.2	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3636RT	0	26.0	0
2694	10^7 3616RT	9x7x2	25.0	10x8x4
2683	10^7 3616RT	0	25.8	0
2684	10^7 3636RT	0	23.8	0
2685	10^7 3616RT	0	27.2	0
2686	10^7 3616RT	0	22.2	0
2675	10^7 8996RT	5x4x3	29.7	4x3x2
2676	10^7 8996RT	6x6x4	24.8	5x5x2.5
2677	10^7 8996RT	0.5x0.5x0.5	25.7	0.5x0.5x0.5
2678	10^7 8996RT	1x1x0.5	30.5	0.5x0.5x0.5
2671	10^7 8996RT	0	24.8	0
2672	10^7 8996RT	0	28.3	0
2673	10^7 8996RT	0	27.6	0
2674	10^7 8996RT	0	25.5	0
2695	10^7 8996RT	7x5x2	26.2	8x6x3
2696	10^7 8996RT	9x6x3	23.4	5x3x1.5
2697	10^7 8996RT	13x11x6	27.0	15x10x7
2698	10^7 8996RT	15x15x5	20.0	15x15x6
2719	3616	0	25.3	0
2720	3616	10x10x7	27.3	11x10x6
SAC 2721	3616			
'95 SAC 2722	3616	19x21x10	—	
SAC 2715	HSV/ GAN			
2716	HSV/ GAN	21x18x8	22.9	23x18x10
9/7/95 SAC 2717	HSV/ GAN			
~~2718~~	~~HSV/~~ GAN			
2735		18x12x8	29.7	16x11x6

Data Sheet

		9/7/95		9/11/95
~~27[illegible]~~	~~HSV/GAN~~			
2731	HSV/RT/GAN	3x2x1.5	25.6	3x2x1.5
2733	HSV/RT/GAN	5x5x1.5	25.8	0
2732	HSV/RT/GAN	13x10x8	22.0	12x8x6

Data Sheet

Greg's Mouse	Data	9/11/15		9/15/15
Mouse#	Group	Measurements	Weight	
2687	RT Alone	7x5x2	26.4	6x5x1.5
2688	RT Alone	18x17x7	29.6	20x18x8
2689	RT Alone	6x6x4	30.2	5x5x4
2690	RT Alone	6x4x2	28.1	6x4x2
2679	10^7 3636RT	0	26.3	0
SAC 2680	10^7 3616RT	[illegible]	[illegible]	
2681	10^7 3636RT	16x11x16	21.7	14x13x7
SAC 2682	10^7 3616RT	[illegible]	[illegible]	
2691	10^7 3636RT	0	25.1	0
2692	10^7 3616RT	0	30.8	0
2693	10^7 3636RT	0	26.8	0
2694	10^7 3616RT	10x9x6	24.7	12x10x6
2683	10^7 3616RT	0	26.1	0
2684	10^7 3636RT	0	24.0	0
2685	10^7 3616RT	0	27.6	0
2686	10^7 3616RT	0	22.4	0
2675	10^7 8996RT	5x4x3	29.3	5x5x1
2676	10^7 8996RT	0	25.4	0
2677	10^7 8996RT	0.5x0.5x0.5	25.3	0.5x0.5x0.5
2678	10^7 8996RT	0.5x0.5x0.5	30.1	0
2671	10^7 8996RT	0	25.2	0
2672	10^7 8996RT	0	28.8	0
2673	10^7 8996RT	0	28.0	0
2674	10^7 8996RT	0	26.0	0
2695	10^7 8996RT	7x6x3	25.2	7x5x2
2696	10^7 8996RT	0	23.0	0
2697	10^7 8996RT	14x10x6	21.3	14x11x6
2698	10^7 8996RT	15x13x7	19	16x14x4
2719	3616	0	25.1	0
2720	3616	13x12x5	28.4	14x17 5
SAC 2721	3616	[illegible]	[illegible]	
SAC 2722	3616	[illegible]	[illegible]	
2715	HSV/ GAN	[illegible]	[illegible]	
2716	HSV/ GAN	12x10x5	26.7	12x6x4
2717	HSV/ GAN	[illegible]	[illegible]	
2718	HSV/ GAN	[illegible]	[illegible]	

Data Sheet

			9/14/95		9/18/95
9/14/95	SAC 2735	HSV/ GAN	24 x 21 x 14	24.3	
	2731	HSV/RT/GAN	0.5 x 0.5 x 1.5	26	Ø
	2733	HSV/RT/GAN	Ø	23.7	Ø
	2732	HSV/RT/GAN	11 x 9 x 6	23.2	10 x 9 x 5

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Cytokine
Mouse log

9/95

Mouse #

5001	cytokine study	c.c. (B)	; HSV-1 3616 in (R) [illegible] ; [illegible] ; [illegible] 20 bj ; [illegible]
5002	"	"	; HSV-1 3616 in (R) ; [illegible] ; sac day [illegible] ; [illegible]
5003	"	"	; HSV-1 3616 in R + L ; [illegible] ; sac day [illegible] ([illegible])
5004	"	"	; HSV-1 3616 in R + L ; [illegible] (R) [illegible] ; sac day 7
5005	"	D54	; HSV-1 3616 in (L) [illegible] ; [illegible] (L) ; [illegible] day 1
5006	"	D54	; HSV-1 3616 in (R + L) ; [illegible] sac day 14
5007	"	D54	; "
5008	"	D54	; HSV-1 3616 in R + L ; sac day 1
5009	"		" ; sac day 7
5010	"		"

11/11/85

U-87 culture starting

Purpose: Start U-87 culture c̄ frozen cell line 3Hg(1:20) for injection into mice.

Media: prepare culture medium for U-87 — Cells: U87 3Hg(?) 8/21/85

500 cc DMEM
50 cc FCS (10%)
5 cc pen/strep
5 cc Na pyruvate
5 cc NEAA

Methods:

Place frozen cell aliquot into warm H_2O bath
Transfer cells to 5 ml of media in 10 ml conical flask drop by drop
Spin in centrifuge for 5' at 800-1000 rpm
Aspirate off supernatant c̄ suction
Resuspend cells in 2 ml of media (drop by drop)
Add 2 ml of cells to 3 mls in T-25 (drop by drop)
Place cells in incubator (top slightly loosened)

9/20/95

C-6 (GBM) in nude mice

Received mice from M.J. c C-6 (R) ...

	ear tag	(L) tumor	(R) tumor	
5001	...			...
5002	L			(R) ...
5003	R			...
5004	LR			(L) (R) ...

inject mice c 10ml 3616 ...

Radiation of C-6 nude

only 2 mice could be radiated due to tumor size
radiated for 20 Gy (10'28")
irradiated ... and LR

Harvest C-6 nude mice tumors in 2 mice (day 1)

		(L)	(R)	wt	(L)	(R)
will harvest	5001	11x9x6 mm	12x7x7	Blank	5.11g	5.16 g
201	5002	12x7x7		tumor+blank	5.64	5.33
502				tumor	0.53g	0.17g
30?	5002	19x15x11 m	19x15x11 m	Blank	5.17g	5.14 g
				tumor+blank	7.19	7.12g
					2.02g	1.98g

Day 9 (9/28/95)

days due to tumor size	5003	21x18x16	23x20x16	5.15	5.21
				6.64	9.6
				1.49	2.39

Day 9 (9/28/95)

5004	22x16x15	17x14x14	5.10	5.12
			5.10	5.01
			7.03	6.60

10/3/95

D54 (GBM) in nude mice – cytokines

Received mice from M.D. ē D54 in (R) hind legs

	ear tag	(L) tumor (mm³)	(R) tumor (mm³)
5005	¢	3×6×6 φ	14×7×9 (3616)
5006	L	11×9×6 (3616)	12×16×6 (3616)
5007	R	[illegible] (3616)	[illegible] (3616)

inject mice ē 10 µl 3616 (2×10⁵ PFU) per above

10/4/95

Irradiation of D-54 mice

irradiate 3 mice ē 20 Gy to (R) hind limb

Harvest of D-54 mice tumors

10/5/95

Harvest 5007 on day 1 (10/5/95); 5005 + 5006 on day 2 (10/6/95)

	(L) mm³	(R) mm³		wt (g) (L)	(R)
5007	14×9×8	16×9×9	tube	5.16	5.03
			tube + tumor	5.93	5.51
			tumor	0.77	0.48
5005	13×10×9	11×6×5	tube	5.18	5.22
			tube + tumor	6.30	5.54
			tumor	1.12	0.32
5006	22×15×12	16×12×9	tube	5.18	5.05
			tube + tumor	6.98	6.31
			tumor	1.80	1.26

In Vitro D-59 Cytotoxicity — 10/6/95

need

0 PFU { 0 hr / 96 hr { 60 / 240 } — 1, 2, 3
1 PFU { 0 hr / 96 hr { 60 / 240 } — 4, 5, 6
10 PFU { 0 hr / 96 hr { 60 / 240 } — 7, 8, 9

need 9 plates plus 1 plate
set up 12 plates

1) Trypsinize Cells
2) Count Cells $12 \times 12 \times 10^4 = 144 \times 10^5 = 14.4 \times 10^6$
resuspend in ~~12 ml~~ 6 ml
3) Add 10 ml media/plate and ½ ml cells soln

Infect Plates 4-9 c̄ HSV-1 (1 PFU or 10 PFU) — 10/8/95
~90% confluent
1) Count cells in spare plate
$30 \times 10\ ml \times 10^4 = 3 \times 10^6$
∴ for 1 PFU want 3×10^6 PFU/plate
for 10 PFU ″ 3×10^7 PFU/plate

$[3616] = 9 \times 10^9$ PFU/ml

plates 4, 5, 6
$$3 \times 10^6 \text{ PFU/plate} \cdot \frac{1\ ml}{9 \times 10^9\ PFU} = \frac{1\ ml}{3 \times 10^3} \left(\frac{1000\ \mu l}{1\ ml}\right) = \frac{1}{3}\ \mu l/\text{plate}$$

3 PFU, not 1 PFU — need 3 plates, set up 9 — $9\ \mu l$ virus in 20 ml Bakem

plates 7, 8, 9
$$3 \times 10^7 \text{ PFU/plate} \cdot \frac{1\ ml}{9 \times 10^9\ PFU} = \frac{1\ ml}{3 \times 10^2} \cdot \frac{10^3\ \mu l}{1\ ml} = \frac{1}{3} \times 10\ \mu l/\text{plate}$$
$= 3.33\ \mu l$/plate virus

set up 4 $= 13.33\ \mu l$ virus in 12 ml media

Add virus 11:30 10/8/95 in 3 ml 2% media

In Vivo Cytokine 10/6/95

seed 0 PFU 0.5 g 1; 2 g (6° 2; 24° 3)

0.1 PFU 0.5 g 4; 2 g (6° 5; 24° 6)

10 PFU 0.5 g 7; 2 g (6° 8; 24° 9)

∴ need 9 plates plus 1 for count cells
∴ 10 plates ⇒ try to set 12 plates

1) Trypsinize cells
2) Count cells 60 X 24 X 10⁴ = 14.4 X 10⁶ in ~~[illegible]~~ 6 ml
3) Try to plate 2X10⁶ cells/plate
4) Add 0.5 ml cell soln to 9 ml media/plate

10/9/95

Infect plates 4-9 c̄ HSV-1 (0.1 PFU, 1 PFU) ~~(+1 PFU or 10 PFU)~~
~ 80% confluent
1) Count cells in spare plate 47 47 ~ 50
$50 \times 10 \times 10^4 = 5 \times 10^6$ cells/plate

infect ~~0.1~~ 0.1 PFU (5×10^5 PFU) + ~~10~~ 1 PFU (5×10^6 PFU)

7, 8, 9 titer 9×10^7 pfu/ml — 1 PFU 5×10^6 PFU $\left(\frac{1\ ml}{2 \times 10^7\ PFU}\right) = \frac{2.5}{10}$ ml = .25 ml ~~2×10^7 PFU/ml~~
= 250 μl/plate in 3 ml media
2 ml in 12 ml
750 λ in 9 ml

~~10 PFU 5×10^7 PFU $\left(\frac{1\ ml}{9 \times 10^9}\right) = \frac{1}{2} \times \frac{1}{100}$ ml $\times \frac{1000\ \mu l}{1\ ml}$ = 5 μl in 3 ml~~

~~make 12 ml~~ add 20 μl virus

4, 5, 6 0.1 PFU 5×10^5 PFU $\left(\frac{1\ ml}{2 \times 10^7\ PFU}\right) = \frac{2.5}{100}$ ml $\frac{1000\ \mu l}{1\ ml}$ = 25 λ/pl
75 λ in 9 ml